STUDIES - PHYSIOLOGICAL AND ANATOMICAL - ON SEEDS

by

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THESIS presented for the DEGREE of DOCTOR of PHILOSOPHY

UNIVERSITY of EDINBURGH.

OCTOBER, 1935.

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INTRODUCTION.

The chain of connected processes and transformations which extends from the quiescent seed to the actively developing juvenile plant may be regarded, in general, as the phenomenon of germination. The first and most important of these changes, and the one upon which all the others depend, is the absorption of water. A study of this prerequisite for success in germination therefore seems fundamental to the elucidation of the entire process, more especially as the question of successful germination bears so definitely on many problems of practical agriculture, as well as on aspects of more purely academic interest.

Relevant to this main problem is the question of how substances dissolved or suspended in the water supplied to the seed affect this process. Here again, as in the main problem, points of academic interest arise, but these are overshadowed by more practical questions because of the increasing use of chemical dressings as preventives against the attacks of seed-borne and soil fungi. Another aspect of the problem is the mode of penetration of these added substances into the seed, where some fungus may be located, which the fungicide must reach if it is to accomplish its beneficial work.

This present thesis, therefore, is a report

of studies on the intake of water by the seed, and the penetration of various substances dissolved in the water supplied to the seed and their effect on germination. It must be made clear that the work is, in the main, exploratory in nature for when initiated the whole conception of the initial stages of water intake by the seed had been recast by the researches of Nelson and MacSween on the Broad Bean, Vicia Faba L., published in 1933. Until that time the path of water intake had been defined as taking place through the micropyle, and the general surface of the seed had been regarded as of minor importance in so far as it affected the intake of water. That this conception is not true, at least for the earlier phases (the first six hours approximately), has been proved. The newer conception is that the first stage is the hydration of the testa, which, being of a colloidal nature, absorbs water by imbibition. The hydrated testa is known to be an imperfectly semi-permeable membrane, and it has been suggested that further water intake is by osmosis through this structure. Some doubt still exists as to the exact nature of the substance which acts as the "attractor" of the water through the seed-coat, but, in the main, the steps in the process as outlined by Nelson and MacSween hold good. A subsidiary point of considerable importance brought out by these workers is that each seed must be regarded as an individual,

because when using genetically standardised material it was shown that while the curve of water intake by a seed of Broad Bean had characteristics similar to another seed, yet they differed in their relation to time. These differences may be, and probably are, related to the pre-history of the seed, e.g. maturation, storage, etc. (Hysteresis of the seed-coat colloids probably covers much of this).

While agreeing in the main with Nelson and MacSween, the present worker is of the opinion that the micropyle is not actually closed during the early stages of hydration, but rather that it is non-effective until after a certain time has elapsed, the time varying in the case of different beans. Microscopic sections through the region of the micropyle, when immersed in water, show not a closure of the micropyle, but a widening and opening of the pore. The reason for the non-effectiveness of the micropyle is due to the fact that a large pad of dry, crushed and air-filled tissue is situated immediately behind it, and this must be moistened before the micropyle can become effective. This is the cause of the lag between the time that soaking commences and the time that intake of water and/or solutions through the pore becomes effective. It would appear, therefore, that after the initial swelling of the testa has taken place the micropyle operates to a certain

extent, and may permit of the passage to the embryo of substances to which the seed-coat itself is impermeable.

With these conceptions in mind it may be postulated that the effect of substances in the water supply would be to influence normal germination in three general directions:

(a) The presence of any substance such as a seed-dressing might, on the one hand, slow up the preliminary hydration of the colloidal seed-coat and, later, tend to slow up osmotic water intake by the seed, thus delaying the subsequent processes, while, on the other hand, the dressings might increase the rate of hydration and the permeability of the seed-coat colloids, thus expediting the intake of water with a possible increase in the rapidity of germination.

(b) Mercury, alone, or chemically combined as in many modern seed-dressings, is deleterious to young living tissue, and the introduction of mercurials into the seed, and their contact with the young embryo, might be harmful, or even lethal. Therefore the mode of entry, and the time at which the mercury reaches the embryo becomes a pertinent

question. The same type of question is raised by the belief that certain metallic radicals, other than mercury itself, have a stimulating action on germination, and that they might be used to expedite germination, and to increase the chances of success of the process. In short, the practical question that emerges is whether mercury and other ions could be applied to the seed under such conditions of control that fungi would be eliminated, while the deleterious effect on the embryo would be evaded and stimulative action obtained.

(c) Given that substances added to the water supply can affect germination, will the stimulus induced be carried forward so as to influence the further development of the plant, and will it be strong enough even to affect the next generation.

MATERIAL.

In the course of the investigation various dyes were employed as it was found that the migration of the coloured molecules and ions in the seed-coat could be followed with a precision not possible when colourless salts are used.

The material used throughout the whole of the studies consisted of a large sample (1 cwt.) of Broad Bean, Vicia Faba L., of the variety known as "Cropper". This material was chosen for the following reasons. The Broad Bean has one of the simplest seed-coats available, and the variety (supplied by the raisers, Messrs.David Bell Ltd.) is as near to being genetically pure and uniform as could be obtained in the quantity required.

While stressing the uniformity of the material and the simplicity of structure it must be remembered that these are only comparative, especially when such a fine analysis as is here attempted is involved. Such commercially pure material doubtless varies genetically and physiologically for the characters involved in these studies, and even with fine sampling methods, and the use of large numbers per test, the individuality of the seed can not be entirely discounted.

The bulk of the seed was received from the growers immediately after harvest in the autumn of 1933, and was kept in the sack in which it was received, in a cool room with a stone floor, samples being drawn for tests as required. Storage in bulk prevents excessive drying out, and the seeds do not reach air humidity, but tend to maintain a stable character, colloid hysteresis and other changes being

to some extent overcome. Storage under perfectly controlled conditions of temperature, humidity, etc., would have been preferable, but, unfortunately, such conditions were not procurable.

MORPHOLOGY and ANATOMY.

A review of the general morphology and anatomy of the seed and seed-coat, as seen in Vicia Faba, is necessary in order that the theories and explanations to be put forward in the course of this paper may be made clear. An additional reason for this review of what is known of the seed from the work of others is that certain structures, not previously described, have now been discovered.

On examining the exterior of a dry seed of the bean the area which stands out most conspicuously from the remainder of the surface of the coat is the hilar scar of hilum (Fig.1). This scar, left when the matured funicle becomes detached, appears as an elongated, oval area, black or dark brown in colour. Extending longitudinally, almost along the whole length of the scar, is the hilar slit, which, in the dry seed, is an open cleft, exposing the underlying tissue. This slit varies in width depending upon the external conditions, particularly humidity: it is completely closed when free moisture is applied to the scar. At the end of the scar nearest the embryonic root is

located a small aperture, the micropyle, to some extent also connecting the external atmosphere with the underlying tissue. At the end of the hilum, distal to the micropyle, a vascular bundle runs through the tissue of the testa. This was described by Beck (1878). This bundle, doubtless, was continuous with the bundle in the funicle, and was probably the path along which water and food were conveyed during the development of the seed in the pod.

On the side of the seed opposite to the embryonic root there is seen a small, brown, slightly raised area, the strophiole (Fig.2). While this strophiole has been considered of some importance in the seeds of other Leguminosae, e.g. in species of Trifolium and Melilotus, not much attention has been paid to it in the case of Vicia Faba.

Turning now to a consideration of the more detailed anatomy of the seed-coat, the first description is that of Bischoff in 1833, and this has been supplemented at different times by other workers, such as Pringsheim (1848), Sempolowski (1874), Beck (1878), and Mattirolo and Buscalioni (1892). The latter added to the anatomical description some explanation of the probable physiological functions of a number of the structures observed by them. In 1899, Pammel published an extremely wide and detailed

treatise on the seeds of the Leguminosae, including Vicia Faba. Naturally, with so many investigators working in different places and at different times a varied terminology has been evolved, and identical structures have been described by different terms. While mentioning some of the names and the alternatives used for parts of the seed-coat in this introduction, the writer, for the sake of uniformity and simplicity, will use only one set of terms in the main body of the thesis.

The seed-coat of Vicia Faba is constructed of several distinct layers of tissue (Fig.3). The outermost layer is one cell deep, and has been named, variously, palisade, epidermal or Malpighian layer. The cells of this layer are longer than broad, with their long axis perpendicular to the surface of the seed, and have blunt or rounded ends. A translucent line, the so-called light line, runs across the long axis of the cells, at about their apex, and appears to divide each cell into two unequal parts. Externally, this palisade layer is differentiated into two more or less distinct strata, the cuticle on the outside, and, lying immediately beneath, the cuticular stratum.

The cuticle consists of a delicate line of almost equal thickness, and may be removed, or otherwise damaged, by being rubbed against surrounding

objects. It has the property of preventing the passage of water, or of allowing it only with difficulty.

The cuticularised stratum is distinguished only with difficulty from the normal cellulose wall of each palisade cell, and is not necessarily composed of the same substance as the cuticle, although named the cuticularised layer. Pore-like canals, which extend into the cellulose portion of the cell-wall, are found on the inner side of the cuticular stratum. These canals may extend from the surface and connect with the cell cavity below the light line.

The walls of the palisade cells are not uniform in thickness, the cell-cavity being somewhat pear-shaped, broader at the inner or lower end, and narrower in the upper part. Viewed vertically from the surface the palisade cells appear to be five or six-sided, with a cavity varying in size according to the depth at which it is focused. Radiating outwards from the cavity in the upper part, through the thickened cell-wall is a number of pore-like canals, which are probably remnants of the original cell-cavity, left as the cell-wall became thickened. A certain amount of protein matter (protoplasm), in which chromatophores are embedded, is found in the cell-cavity, and some tannin is also present.

The most interesting feature of the palisade

cell is, without doubt, the "light line", regarding the function of which there has been much speculation, and many theories have been put forward to explain its physical properties and chemical composition. Russow (1873) concluded that in it the cellulose of the wall had a modified molecular structure, containing less water than the remainder of the cell wall. Sempolowski (1874) states that there is not only a difference of molecular arrangement, but also a chemical modification of the cell-wall at this point. Beck (1878) showed that the "light line" had small affinity for water, and he thought that its appearance might be due to chemical alteration though microchemical tests failed to reveal its nature. Harz (1885) accepted Russow's explanation regarding the line, and added that it was entirely due to physical changes in the laying down of the cell-wall substances, and that it did contain less water. The noteworthy absence of pore canals in the region of the "light line" certainly does cause it to appear denser, while its contact with the neighbouring porous parts of the wall intensifies its lustre. Coe and Martin (1920) maintain that the "light line" is pierced by closed canals, which are opened on hydration by mechanical stretching due to imbibition, while Hamly (1932) states that the "light line" in the seeds of Alfalfa is situated at the junction of the suberin caps of the

palisade cells and the ordinary palisade cell-wall.

At present there would appear to be no satisfactory explanation of the chemical or physical constitution of the "light line", while its function still remains obscure. Mattirolo and Buscalioni (1892) state that the "light line" checks transpiration during dry weather: it does not prevent water from entering, but checks its outward flow. The outer part of the palisade cells, beyond the "light line" and below the cuticle, is mucilaginous and takes up water, whereas the "light line" allows but little water to pass and swells only slightly, although it is passively stretched and, in this way, the canals become enlarged and permit water to pass. This is similar to the theory of Coe and Martin that the seat of impermeability in seeds of Sweet Clover is the "light line". Canals cross this line and they require to be stretched before they will allow any water to pass.

The position, then, would appear to be that there are a great many opposing views concerning the subject of the "light line", and no definite, agreed theory regarding it has been formulated.

Immediately underlying the palisade cells and almost invariably accompanying them are the osteosclerids or hour-glass cells. Where the palisade layer curves in at the hilum these particular cells become first larger, and then shorter, and merge into

the star-shaped parenchyma of the hilum. The walls of the hour-glass cells are greatly thickened and frequently possess lateral processes, and in some areas the cells are marked with longitudinal canals. The inter-cellular spaces between the cells are prismatic in outline.

Beneath the layer of hour-glass cells is the nutrient layer, which may be two or three cells deep, the uppermost cells having thin walls, and the lower cells thick walls. In the developing seed this layer contains both chlorophyll and starch, the latter possibly serving to nourish the growing embryo. As the seed approaches maturity the cell-walls of the nutrient layer collapse, and the cell cavity then appears only as a thin line in the dry seed. When the mature seed is soaked, however, the cavity reappears.

The vascular bundle, already mentioned as beginning at the hilar scar, is continued into the nutrient layer along the raphe, and just before it reaches the embryo it bifurcates. The two branches thus formed subdivide further, and gradually lose their identity when they come into close proximity to the embryo. The vascular bundle itself is composed mainly of xylem elements.

A brownish pigment is found in the cells of the nutrient layer, and associated with it is a quantity of pyrogallol tannin. It has been shown by

Brown (unpublished) that although the amount of tannin varies considerably as between different seeds of the same sample it is always present in an appreciable quantity.

Of the differentiated areas of the seed-coat the most prominent is the hilar area and its anatomy is not without interest (Fig.4). A section cut across the hilum shows a double layer of palisade cells, the long axis of the cells of the outer layer shortening towards the edges of the hilum, the inner shortening towards the tracheid island. A light line occurs in both layers. The hilar slit or groove is seen to be connected with the micropyle and runs the entire length of the hilum. This slit extends in depth through both of the palisade layers, and the underlying tracheid island is thus exposed to the external atmosphere.

The tracheid island is somewhat ovoid, with pointed ends, and it consists of a bundle of tracheidal cells, surrounded by several rows of thin-walled elements. The amount of parenchyma increases in the hilar region and may be differentiated into three regions, (a) thin-walled parenchyma forming a continuation of the nutrient layer, (b) thicker-walled, star-shaped parenchyma with numerous large intercellular spaces, and (c) thin-walled, elongated elements surrounding the tracheid island.

The function of the tracheid island in the

mature seed is difficult to explain. Mattirolo and Buscalioni showed that water passed through the hilar groove, but the present worker has found that the hilar groove closes as soon as it is moistened, and therefore, as has been suggested by Pfaefflin (1897), the amount of water passing through must be limited. The micropyle is also hygroscopic in its action, but it appears to open rather than close when moistened.

The radicle of the embryo lies in a pocket of the nutrient layer in close proximity to the micropyle, but always above it. This pocket, or jacket, fits closely round the radicle, especially at the upper end where the radicle merges into the hypocotyl which is joined to the cotyledons. The plumule lies between the two cotyledons and is protected by them.

The strophiole, which is situated on the seed-coat at the side opposite to the embryo, is a particular area in which the palisade cells are narrow and much elongated, gradually shortening and broadening until they merge into the normal palisade tissue on either side of the protuberance. The hour-glass cells in this area are also slightly longer, and the intercellular spaces narrower.

The particular function of the strophiole is not at present known, but cracks occur frequently between the palisade cells in the strophiole, allowing

water to pass through. It is suggested that in a seed like the Broad Bean the strophiole is no longer functional, but in a seed the coat of which tends to be impermeable to the passage of water, these cracks would serve to render the coat permeable and so allow the seed to swell and germinate.

While studying the anatomy of the seed-coat of Vicia Faba, a peculiar structure, which does not appear to have been previously described, was discovered. At the edges of the pocket enclosing the embryonic radicle two dark-brown, half-moon shaped bands are visible on the seed-coat (Fig.5). These bands lie on the innermost face of the nutrient layer and fit closely into the lateral sides of the radicle in the hypocotyl region. They differ from the neighbouring tissue in having a shiny appearance, and in being slightly raised above the level of the surrounding tissue: when studied in surface view it was observed further that these bands have the appearance of a honeycomb or network, with raised walls and sunken cavities, as distinct from the smooth surface of the adjacent tissue (Fig.5a). Microchemical tests of the tissue of the band indicated that it is of a cellulose nature.

When those parts of the hypocotyl in contact with the bands were examined shallow hollows were found on the surface, similar in shape to the bands of

the coat, and on microscopic examination the cells in each sunken area were seen to be papillate, projecting slightly, with depressions between the cells, indicating that these projecting cells would fit closely into the corresponding sunken cavities of the inner layer of cells of the seed-coat.

Sections were cut through the bands, with the embryo in its normal position, and it was then confirmed that the cells of thecsunken hypocotyl region did in fact fit closely into the raised bands of cells on the inner face of the seed coat, the ridges on the coat fitting into the hollows on the hypocotyl and vice versa (Fig.6). No little difficulty was experienced in keeping the root and seed coat in situ during the process of fixing, cutting and staining, even with material embedded in paraffin wax, on account of the different rate and amount of dehydration of the seed coat as compared with the tissues of the embryo. For this reason sections were also cut of the two tissues separately. From both series of sections it could be seen that the flat-surfaced cells of the cotyledon passed over into the papillate cells of the hypocotyl and then into the smooth surfaced cells of the root (Fig.7).

Where the papillations were most marked about one half of each cell projected out from the point where it was joined to its neighbouring cells.

The tissue of the band on the inner surface

of the seed-coat in section formed a serrated edge to the nutrient layer in comparison with the neighbouring more or less smooth surface (Fig.8). The serration consisted of thick projecting walls, tapering slightly to the tips, and sunken cavities. The projecting walls fitted in between the cells of the hypocotyl region, locking the tissues of the coat and hypocotyl firmly together in that area.

At once the question arises - what is the nature of this structure and what purpose does it serve? At first it was thought from the general appearance of the structure, and the close contact that it formed between the nutrient layer of the coat and the embryonic tissue, that it had been a means of conveying food from the nutrient layer to the embryo. Colour was lent to this idea from the fact that the cells of the hypocotyl and cotyledon appeared to be elongated from the papillate area in the direction of the plumule. This path of elongated cells would possibly act as a path for the easy diffusion of food through the tissue to the plumule (Fig.9). The idea behind the theory was that this structure was intended for the rapid transport of food from the nutrient layer to the embryo proper, in order to build up a concentrated food reserve in the embryo during maturation.

In order to verify the theory, young immature

beans were fixed and embedded in paraffin wax and sections cut through the area on which the bands were normally found in the mature bean, but, unfortunately, little or no trace of the bands could be seen in the immature beans (Fig.10). This indicated that if the bands were intended for the transferance of food such movement must take place late in the history of the development of the bean embryo.

Further examination of the sections of the immature bean showed that some of the last remnants of the true endosperm were lying between the nutrient layer of the coat and the embryo, in that area in which the bands would eventually develop.

The discovery of these facts rather threw a doubt on the theory of the bands acting as a means of food conveyance, and the sections of the mature bean, as well as the beans themselves, were re-examined with the view to discovering what other function this structure might perform. In this second examination it was seen that the surface of the papillate cells on the hypocotyl was cutinised, a fact which further tended to disprove the theory of food transferance. Having regard to the structure of the bands, and their position just at the end of the pocket enclosing the radicle, it was then suggested that these might act as clamps, holding the root in position and preventing the embryo from altering its position during the drying

or soaking of the seed. When the radicle begins to elongate and to swell during the first stages of germination, if it were not fixed at its base by these clamps, it would be prevented from growing forward by the mechanical barrier of the coat, and hence a pressure would be set up backwards which would put a strain on the hypocotyl region. The cotyledons being unable to move, this strain on the hypocotyl would cause the latter to rupture, thus separating the root and the plumule from the cotyledons, and leading to the production of what is known in the terms of official seed analysis as a "broken seedling" (Nelson, 1924). The purpose of the bands therefore is to act as a clamp, holding the radicle and forcing it to grow forwards so as to rupture the coat, not <u>at</u> the micropyle as laid down by many authorities, but <u>above</u> the micropyle as normally happens. The coat acts naturally as a barrier to the root because it is tough and, comparatively speaking, some considerable force is required to rupture it. If the same force were applied to the much more delicate hypocotyl, which is mainly composed of parenchyma, there is no doubt whatever that it would break. Therefore if this clamping mechanism were not present to prevent this backward pressure being exerted on the hypocotyl a breakage in this region would be a frequent occurrence.

Attempts were made to prove the necessity for

these clamping bands during the first stages of germination. A portion of the coat and the band immediately below were removed from dry beans without causing any injury to the actual embryo itself. These beans, so treated, were soaked and germinated, and in almost every case there was rupture of the hypocotyl, not a complete break but sufficient to bring about a slowing down of the food-transference, while permitting also the easy access of fungal and bacterial diseases. It is not suggested that these experiments are by any means conclusive as abnormal conditions were set up by the removal of the coat as well as the band. The ideal would be to remove the band without touching the overlying coat, but this is obviously impossible.

While the development of the bands has not been fully worked out as yet, it would appear that in the later stages of maturation the remnants of the endosperm are forced against the nutrient layer by the enlarging embryo, causing a close contract to be made between the nutrient layer and the endosperm. The pressure exerted is no doubt sufficient to cause the endosperm and some of the nutrient layer tissue to be forced down between the cells of the hypocotyl, thus forming rudimentary bands. The subsequent hardening of the nutrient layer and the remains of the endosperm would strengthen the bands, and thus the

papillate tissue on the hypocotyl and the honeycomb network on the nutrient layer are brought about.

At this stage it may be concluded that these dark brown bands on the nutrient layer of the seed-coat are honeycomb-like structures developed to fit into a corresponding papillate area on the hypocotyl. The function of the combined structures is to hold the radicle in position during the early stages of germination, thus preventing a rupture of the tissues of the hypocotyl due to the backward pressure set up by the radicle in its endeavour to break through the mechanical barrier of the seed-coat.

How far this theory may be applied to other Leguminosae is not certain, but it seems not improbable that similar bands will be discovered in the coats of other seeds. The absence of these bands, or their non-functioning, might account for the broken seedlings found during germination tests of many samples of leguminous seeds. In such "broken seedlings" the break occurs just at the point where the hypocotyl joins the cotyledons, which, as suggested by the author, is the point where a break would occur if these bands were not present, or were non-functioning.

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THE PENETRATION OF DYES THROUGH THE SEED COAT OF VICIA FABA L.

Dealing with the physiological aspect of the problem it will be most convenient to consider first the work on the penetration of dyes through the seed-coat of Vicia Faba, as some of the facts discovered during the experiments with dyes serve to clarify the work on the mercurial salts. The aim of these experiments was to discover the path of entry of a coloured substance through the seed-coat, and later, by analogy, that of the colourless salts.

In all cases here reported 1% solutions of the dyes in distilled water were used. As exposure to light on standing may produce some change in the solutions these were freshly made before each experiment. Groups of from twenty to twenty-five beans were soaked in the respective solutions, contained in cylinders with ground-glass stoppers, at ordinary room temperature. In every case the beans used were carefully selected by hand to ensure that cracks visible to the eye were absent from the coat at the commencement of each experiment. Even with the greatest care beans with very minute cracks were sometimes passed, but such seeds, when discovered later during the course of the experiment, were discarded. At two-hourly intervals the beans were removed from the solutions, and were washed and examined. At the time when this work with dyes was undertaken the beans were

in such a state of maturation that soaking for twelve hours was sufficient for the complete swelling of almost all the seeds in the test. With beans aged by storage, a longer period is necessary. At each test one or two typical or average beans were examined, and the particular areas of the surface of the seed where penetration of the dye had taken place were noted. Portions of the seed-coat were then removed from different areas of the bean, and the outer surface of the coat was examined under a microscope; the mounting agent employed here was heavy-bodied paraffin oil, which, while making a comparatively permanent mountant, also acted as a clearing agent. The particular areas of these pieces of the coat which had been stained with the dye, and any peculiarities which might be apparent were noted. Transverse sections of similar pieces of seed-coat were also examined, and in these cases the depth of penetration of the dye in the stained areas was observed. In this way it was hoped to make a fairly complete record of the passage of any one dye through the seed-coat of Vicia Faba.

Results of Experiments on Dye penetration.

(1) Basic Fuchsin: With this stain at the end of the first two hours, the beans were little changed in external appearance, apart from normal swelling, but the dye had stained the upper surface of the hilar

bulges and any ridges that might be present on the coat, while isolated patches of dyed tissue could be seen in the hollows. Some beans had not stained at all: such difficulties due to individual idiosyncrasies were encountered throughout all these experiments. Just as it has been noted that some beans absorb pure water more quickly, or more slowly, than the average, so some beans absorbed the dye more rapidly than the majority, while others absorbed the dye more slowly.

Surface preparations showed that small localised areas of the testa had absorbed the dye, while the remainder had not, and a mosaic effect was thus produced. These stained areas occurred in the hollows between the shallow ridges, which can be observed in any bean before soaking, and indicated that certain cells, or groups of cells, were more permeable to the dye than others. Any slight mechanical scratch on the surface of the coat was deeply stained, and the surface of the ridges being more exposed to such mechanical injury might account, to some extent, for their being more heavily stained than the hollows.

Sections of the coat showed that where the dye had penetrated it had passed only a little way beyond the light line. In fact, penetration past this line was rare in the early stages of soaking.

Beans left in the dye solution for a further two hours were slightly wrinkled, and the ridges and bulges were more deeply stained than before. The mosaic of dyed, undyed, or less strongly dyed areas was still visible in the surface preparation, and the more strongly dyed patches appeared to be slightly raised above the surface of the surrounding cells. When the coat was sectioned it was found that the dye had not yet passed beyond the barrier of the light line.

At the end of six hours the whole of the surface of the beans was wrinkled and was patchily red all over. The ridges and bulges, however, still showed a slightly deeper stain. In the surface preparations the mosaic now consisted of dark-red and lighter stained areas, with few unstained patches. When sections of the deeply stained areas were examined it was discovered that the dye had now passed the light line, and that diffusion had taken place into the upper half of the palisade cells.

At the end of the fourth interval (eight hours staining) the beans were well wrinkled, and only in the hollows were unstained areas to be found. That the dye was still collecting in definite areas, and also in the scratches on the coat, was apparent in the surface preparations. In sections, the dye was now seen to have passed into the lumina of the

palisade cells, but no lateral diffusion from stained into unstained areas of the coat had taken place. A section cut down through the micropyle showed that the underlying nutrient layer, and the hour-glass cells for a short distance behind it, were stained red, while the dye had not yet passed right down into the palisade cells in that area. This indicated that the micropyle was open, and was allowing the dye to enter.

After ten hours, some of the beans were still wrinkled, but in others the wrinkling stage was passing off. The dye was still mainly concentrated on the ridges and bulges, and the mosaic of more deeply and more lightly stained areas was still visible on the surface preparations. Sections demonstrated that the dye was passing into the hour-glass cells in the more deeply stained areas, but unstained areas were still visible on the bean coat in the sections, and no diffusion into these, either from outside, or laterally from the deeply stained neighbouring cells, had taken place. Sections of the micropyle area again showed a slight penetration of the dye into the nutrient layer and hour-glass cells but it had not penetrated far.

Some of the beans were now completely swollen and were soft to the touch, and these had the dye over nearly all the surface: the dye was found to have penetrated even to the nutrient layer.

At the end of the twelve hour period the majority of the beans were swollen and soft while others were still in a wrinkled condition. Little can be added to the observations made during the previous period except that on some of the wrinkled beans there appeared to be a fading-off of the concentration of the dye from the top of the ridge to the hollow. Again, little diffusion of the dye from the micropyle was found except for a short distance in the nutrient layer and hour-glass cells.

During this period of soaking no penetration of the dye into the cotyledons or embryo proper had taken place, except in the case of a few beans where a minute crack in the strophiole had allowed direct contact between the cotyledons and the solution. The dye in these cases had passed round, under the coat, and was absorbed by the nutrient layer and passed into the cotyledons and embryo. With prolonged soaking in the case of normal beans without any cracks on the coat the dye did eventually pass into the surface layers of the cotyledon and embryo.

(2) Miscellaneous Basic Dyes: Similar experiments were conducted with other basic dyes, e.g. Nile Blue, Janus Green, Neutral Red and Methylene Blue. In all cases the phenomena observed were the same as those described for Basic Fuchsin, although in the case of

Janus Green the passage of the dye through the coat appeared to be slower.

With Methylene Blue the mosaic of stained and unstained areas was even more remarkable than with Basic Fuchsin, and in some cases isolated cells appeared to have absorbed the dye more readily than others and presented the appearance of dark blue dots on the surface preparations. The amount of diffusion from the micropyle into the underlying tissues was slightly greater, and a slight apparent diffusion from the hour-glass cells into the palisade cells was visible.

(3) Acid Fuchsin: This was the first of the acidic dyes to be considered because of its being in direct contrast to Basic Fuchsin.

At the termination of the first two hour interval the beans were practically unchanged as regards swelling. The dye however had concentrated on the ridges, and, to a lesser extent, on the hilar bulges. In surface preparations a definite mosaic of dyed and undyed areas was visible all over the coat. When a number of the small dyed areas occurred closely enough together a patch, visible to the naked eye, appeared as a stained area. In sections the dye appeared to have been absorbed only by the outermost surface of the coat. Naturally light-coloured seeds appear to absorb more dye than naturally dark-coloured

beans, but this is not to be relied upon as after all it may be due to an optical effect, brought about by the more striking contrast between the light coat of the seed and the dye.

After four hours some of the beans showed the first signs of wrinkling, but little or no change was visible in the amount of dye absorbed. The dye was still localised on the surface of the coat, although in some cases the dye had stained the coat with a network of thin lines which took the shape of the outline of the palisade cells.

On the completion of the third interval it was noticed that while the majority of beans were wrinkled, and only partly stained, two were completely red all over the coat.

In the majority of the beans most of the surface was tinged with red, with an aggregation of the dye in certain areas. In sections the dye was seen to have penetrated as far as the light line.

The totally red beans were examined and the micropyles were found to be wide open, all the underlying tissue having been stained red - an indication that the dye in this case had passed through the micropyle into the nutrient layer and hour-glass cells, and from these right round the coat and up into the palisade cells.

At the end of eight hours the state of the

beans was very much the same as before, only the wrinkling was more pronounced. The dye appeared to be concentrated more on the ridges and bulges, probably due to mechanical absorption in the scratches which are most frequent in those areas. Two of the beans were completely red and one was red on the hilar bulges and periphery. In all three the micropyle was open.

In the normal beans the dye was still confined to an area outside the light line although in a few places there was a very slight indication of penetration into the palisade cells.

Of the bean which was stained half red, sections were cut through the junction of the stained and unstained area. There was an indication of rapid diffusion in the hour-glass and nutrient layer cells, and from these into the palisade cells. The dye in the hour-glass cells was slightly ahead of the stained palisade cells and there was a diminution of the dye outwards in the palisade cells from the hour-glass cells.

On the completion of ten hours soaking the dye was still situated mainly on the ridges and bulges. Surface preparations showed mosaic areas of dyed and undyed cells with deeper red patches on the surface of the ridges. On some of the ridges, sections showed penetration into the palisade cells.

Some of the beans were totally red, except in a few areas which were still hard and closely pressed against the cotyledons. The same traces of lateral diffusion into these areas was visible as before.

At the end of twelve hours the majority of the beans were completely swollen, others were totally wrinkled. The soaked beans were completely red while the wrinkled beans were still only slightly stained. The dye was mainly concentrated outside the light line, and in only a few cases had it passed that point and had penetrated into the palisade cells.

Little or no diffusion of the dye into the cotyledons was visible, but after prolonged soaking diffusion did take place.

Other acidic dyes, such as Indigo, Carmine, Light Green and Congo Red, were offered to the beans and in each case similar results were obtained, the dye appearing to pass through the micropyle.

Congo Red was of particular interest because while it passed round the inner coat mainly in the hour-glass cells it did not pass out into the palisade cells. In some cases the dye did not succeed in passing the whole way round the coat, but only so far as the hilar bulges and round the periphery of the coat for a short distance. In some cases the colonnades of the hour-glass cells were the

only parts of the tissue which were stained in the actual coat, as distinct from the hilar area.

In beans in which the micropyle had been plugged with wax prolonged soaking did allow the Congo Red to pass through the coat, indicating that the swollen testa is not totally impermeable to the dye, the plugging of the micropyle preventing the access of the dye to the tissues through the pore. In some cases beans with unwaxed micropyles also showed a penetration of the testa by the dye, but here the seed-coat was completely hydrated.

In a few of the beans examined the Congo Red, in the early stages of soaking, had entered the bundle, situated at the end of the hilar slit, and had passed in the bundle round the coat for a considerable distance, but no diffusion from the bundle into the coat was discovered. This may indicate that the bundle could act as a path of water intake into the bean in certain cases.

The effect of soaking seeds of Phaseolus vulgaris and Pisum sativum in Congo Red was also observed. The results were the same as those obtained with Vicia Faba, the dye passing in through the micropyle and round the nutrient layer and hour-glass cells.

Further experiments were carried out with the basic dyes on beans in which portions of the seed-

coat had been waxed, the object being to discover how far lateral diffusion takes place in the seed-coat layers, other than the nutrient layer and hour-glass cells. It was found that lateral diffusion, if any, is negligible, for sections cut through the junction of the waxed and unwaxed areas showed a sharp line of demarcation between the dyed, unwaxed portion and the undyed, waxed area.

Attempts to discover the cause of the difference in permeability of the cells to the dye, evidenced by the mosaic effect, proved futile. No microscopical difference was visible, and no microchemical distinction could be found. The essential difference between these cells must be extremely fine.

A test of the germination power of beans soaked in various dyes was carried out, and although the beans were deeply coloured the development of the embryo seemed to have been in no way impaired. This is of interest as it shows that mere impregnation of the seed-coat with substances need not have any ill effects on the enclosed embryo. In some cases migration of the dye from the testa to the developing embryo was observed, but no detrimental effect on the plantlet was seen.

It is apparent that the dyes employed may be divided into two classes according to their mode of

entry into the bean. The two classes coincide with the dye classification of acidic and basic. In the acidic group the dye does not pass through the coat in the early stages of swelling, but when the coat has become wrinkled as the result of unequal hydration the dye can pass through the coat, although the light line constitutes a partial barrier. While there is no general passage of the dye into the coat in the early stages of swelling, yet there are certain cells of the coat which appear to possess a greater affinity for the dye, or are slightly more permeable to it, than others. When the micropyle opens, however, these acidic dyes pass in through it, and travel round through the nutrient layer and the hour-glass cells. In the case of Acid Fuchsin there is a diffusion outwards from the hour-glass cells into the palisade cells, but this vertical spread does not happen in the case of Congo Red. The dye also passed round between the cotyledons and the actual seed-coat itself. Any small cracks in the coat, such as are frequently found at the strophiole, allow the dye to pass in almost immediately, or after a very short interval of soaking.

On the other hand, the basic dyes immediately "adhere" to the coat particularly in any scratches and in any definite localised areas. "Adhesion" to the injured testa is probably mechanical, but in the case of the localised areas it must be due to a greater

affinity for the dye, or else the cells are more permeable than others, although no anatomical or microchemical differences could be found. The dye passes through the coat, particularly in these localised areas, until it reaches the light line which would appear to constitute a partial barrier to the dye. Further stretching of the coat, due to continued imbibition of water, allows the dye to pass into the coat more generally, but the cells which started to absorb the dye first still allow the dye to pass through more rapidly. These cells retain the initial start then obtained due to their slightly greater permeability in the very early stages of swelling. The barrier offered to the dye by the light line is broken down by the swelling or stretching of the coat; and the dye passes right through the palisade cells and hour-glass cells into the nutrient layer. The dye did not appear to pass into the embryo or cotyledons, at least within the period of the experiment, but with prolonged soaking the dye did penetrate into these structures. When the micropyle does open to allow the basic dye to enter, the subsequent history is dissimilar to that of the acidic dyes, the basic dyes being held localised round the young radicle, adjacent to the micropyle. The failure of the basic dye to spread from the micropyle is explained by experiments carried out with extracts from the seed-

coat.

The coats of several beans were removed, and these were placed in distilled water and allowed to stand for a short time to extract the pyrogallol tannin which is present in the seed-coat. The solution was then decanted carefully, and when mixed with the basic dyes a distinct, coloured precipitate was produced. It is suggested that this is what actually occurs in the hour-glass cells and nutrient layer cells, the locus of the tannin in the natural seed. The hypothesis being that the dye passes in through the micropyle and is precipitated by the tannin, and, as more dye flows in from behind, it is in turn precipitated, gradually forming a plug of precipitate which blocks further passage from the micropyle into the coat.

While the tannin in the palisade cells and hour-glass cells no doubt also precipitates the dye in its passage through the coat, there is not a sufficiently dense precipitate formed to block the pathway of entry of the dye. Just behind the micropyle, in the nutrient tissues, there appears to be a concentrated mass of tannin, which no doubt would intensify the blockage there.

When the tannin was cleared from the testa extract by precipitation with gelatine, the addition of the dye to the resultant liquid produced no

precipitate, thus confirming the view held as to the action of the tannin on the dye.

Acidic dyes produced no precipitate when added to the water extract of the testa.

DISCUSSION.

The first interesting point that arises when considering these results obtained from work with dyes is that the hilar bulges and the ridges of the seed-coat appear to be more permeable to dyes than the corresponding hollows in the coat. Paine and Saunders (1918), working with wrinkled peas, discovered that dye penetration took place only on the wrinkles. This was due to a waxy bloom in the hollows in the coat preventing the entry of the dye. This bloom had been removed from the ridges by mechanical rubbing against surrounding objects. This explanation would probably apply also to beans, the slight waxy covering or cuticle on the coat having been removed from the hilar bulges and ridges by rubbing, thus rendering them more permeable to the dye, although in this case the dye did not penetrate into the cells in the hollows. Why even on the ridges some cells should be more permeable than others can hardly be explained on this basis, although it may be due to the removal of more, or all, wax from the more permeable cells, as compared with the less permeable cells. The same might apply

to the hollows, the more permeable cells having a thinner cuticle or waxy coating. Braun (1924) showed that the distal end of the wheat grain was more highly cutinised than the embryo end, indicating a cytological basis for the gradient of permeability to iodine which he had discovered. The same phenomenon ought to apply to cells or groups of cells in the bean coat.

The next barrier to the passage of dye through the coat in the early stages would seem to be at the top of the palisade cells, in the region of the light line. The dye appears to pass through the cuticular and subcuticular strata fairly readily, but is stopped at, or about, the light line (Hamly, 1932). Coe and Martin (1920) stated that the light line formed the impermeable region of the seed-coat in sweet clover seed, and that this region was pierced by canals which were closed, but as swelling took place the pores opened and permitted the passage of dyes. Lute (1928) also stated that alfalfa seeds become more permeable when the tips of the Malpighian cells are planed off. This would also indicate that the semi-impermeable region of the coat lay at the top of the palisade cells. Hamly maintains that the impermeability in Melilotus is due to the presence of suberin caps on the Malpighian cells, the junction of these caps and the true palisade cell-walls being at the light line. These suberin caps, according to him,

are closely pressed together and prevent the entry of water into the seed, and, further, the opened canals in the light line in soaked seeds, described by Coe and Martin, are normally present in the unsoaked seed but are not visible. It is important to note that Hamly worked with "hard" seeds which are not found in the case of the bean, consequently some further mechanism must exist which overcomes the impermeability of the coat. Brown (1932) in his investigations on wheat maintains that the mechanism for overcoming the impermeability of the wheat grain coat is by the intake of water by the micropyle, causing the endosperm to swell and the coat to stretch, thus opening up the intramolecular spaces in the colloid, and allowing substances to pass through. These findings by Brown require clarification in that the structure called the micropyle is not properly defined. A caryopsis does not possess a true micropyle.

In the case of the bean, after an interval of time, some change takes place in the region of the top of the palisade cells, rendering them permeable. Collating the views of Brown and Coe and Martin, it appears that a certain amount of imbibition of water by the colloids of the coat surface takes place, causing them to swell and to open up the intramolecular spaces in the colloid, and opening also

the canals into the cavities of the palisade cells by the stretching of the coat, thus permitting the dye to pass into the coat. Where the actual stoppage of the passage occurs is not quite clear, but it is somewhere in the region of the apex of the palisade cells.

Pammel describes longitudinal canals which pass across the light line, making connection with the palisade cell cavity, and it is possible that the stoppage may occur at the apex of these canals. It is more probable, however, that the stoppage is made at the light line, as it is the only visible differentiated tissue, a constriction of the canals taking place at this point, and it is necessary for a certain amount of water to be absorbed before the constriction widens sufficiently to allow of the passage of substances into the coat. Once past the light line the dye can pass through the palisade cell canal into the cell cavity and into the hour-glass cells.

The fact that a stretching of the coat is necessary before the dyes can pass would indicate a further reason for the greater permeability of the ridges and any wrinkles that might appear on the coat during soaking. It is reasonable to assume that the cells on the ridges, at least the upper ends of the cells, are already slightly stretched, consequently

the opening of the canals would not take so long as in the case of the cells in the hollows. The same holds good for the wrinkles, although in this case it would be that the canals of the cells in the wrinkles were more widely open and therefore more permeable than the canals of the cells in the hollows.

A fact, previously noted, can be recalled here, namely that the cells of the coat which were most permeable to the dye appeared, after soaking for a short time, to be raised above the surface of the neighbouring cells. This would mean that the colloids of these cells had absorbed water more rapidly, and had swollen more quickly, than neighbouring cells, thus, by enlarging the intermicellar spaces, had rendered them more readily permeable to the dye.

Once the passage into the palisade cells is opened the two classes of dyes - acidic and basic - behave differently as regards the early stages of absorption. The basic dyes continue their passage through the coat, the larger moleculed dyes penetrating more slowly into the hour-glass and nutrient layer cells, but the acidic dyes do not enter into the palisade cells. This difference in behaviour of the two dyes remains to be discussed.

A large proportion of the work carried out with regard to the penetration of dyes has been done with living cells and while this is naturally not

homologous with the penetration of dyes into dead tissue such as the seed coat, it is of interest to note that living cells are also more permeable to basic than acid dyes, e.g. Brooks (1933) found that living cells were permeable to basic dyes regardless of their degree of dissociation, but permeable only to acidic dyes when they were weakly dissociated.

Rideal (1926) in his work dealing with surface chemistry gives the clue to the whole explanation. Charged ions or particles of the same sign as the micellae of the membrane penetrate relatively quickly whilst those of opposite sign are precipitated during their course and the rate of penetration is extremely small. The micellae, as has been noted, become hydrated in the dye solution and thus adsorb substances with polar groups whether ionised or not. The rate of penetration through the intermicellar spaces is reduced by this adsorption of the diffusing molecule in the adsorbed water layer round the micellae. Membranes themselves, consisting of a colloidal network, may evidently undergo marked variation in intermicellar, or cross section, free space if the hydration of the membrane be altered, thus affecting their permeability to various dissolved materials.

This can be applied to the seed coat colloids and the walls of the canals of the palisade

cells. If the colloids of the coat and the canals bear the opposite charge to that of the acidic dyes, they will prevent the entry of the dyes into the coat whether it is ionised or not, but later, when total hydration of the coat has taken place, the inter-micellar spaces in the colloids, and the diameter of the canal, will have increased sufficiently to permit of the passage of the dye. This in actual practice was found to be the case - acidic dyes could pass through the coat when it was totally hydrated. Naturally, basic dyes being of opposite charge to acidic dyes can pass through the coat even if not ionised.

Up to the present no mention has been made of the micropyle in the discussion. When the micropyle opens, as it did in the beans the author was using (after six hours), the acidic dyes pass right round the coat. This would indicate that the spaces of the nutrient layer were sufficiently large not to have any effect due to charge, although the fact that the basic dyes are held a short distance behind the micropyle might indicate a change of charge in the nutrient layer; but since the basic dyes can penetrate into the nutrient layer after passage through the coat this alteration of the charge does not seem likely. The explanation put forward during the experimental results is more

probably correct, namely that the dye is precipitated by the tannin lying close behind the micropyle, and this precipitate forms a plug which prevents the further passage of any more dye. The acidic dye is not precipitated and can thus penetrate into the nutrient layer.

The spreading upwards of Acid Fuchsin from the nutrient layer into the hour-glass cells and palisade cells can readily be explained. When the micropyle opens, allowing water to enter, hydration of the coat takes place rapidly from below, the canals of the palisade cells and the intermicellar spaces of the colloid are sufficiently open to allow of the passage upwards of the dye into them, and thus to stain the whole coat. As the water moves round under the coat the dye follows in its wake and moves up into the canals as these are hydrated from beneath.

The precipitation of the basic dyes by the tannin solution is similar to the findings of Scarth (1926) for the accumulation of basic dyes in living cells. Combination of the basic dyes with tannic acid, and then adsorption of this combination to a colloid, was the mechanism of accumulation postulated by him. Something similar may be occurring here, combination of the dye and the tannin producing a precipitate direct, or else a further combination of

dye plus tannin with some colloid, extracted from the coat, is taking place. The first theory seems more probable as removal of the tannin, which is the main constituent of the extract, reduces the precipitate to nothing.

The remaining experiments with the dyes showed that there was no lateral movement of the dyes in the palisade and hour-glass cells as they passed through the coat, only longitudinal movement being visible.

CONCLUSIONS.

1. The charge carried by the colloids of the seed-coat of *Vicia Faba* L. only permit of the entry of basic dyes in the early stages of water absorption, but not of the entry of acidic dyes until total hydration of the coat has taken place.

2. The first barrier to the passage of all dyes is the cuticular or wax stratum on the coat, which is most effective in the hollows where it has not been removed by mechanical rubbing.

3. The second barrier to the passage of the dyes is in the top of the palisade cells in the region of the light line. Imbibition of water and subsequent swelling of the coat colloids and

opening of the palisade cell canal is necessary to overcome this barrier.

4. There are cells on the coat, distributed in more than one area, which are more permeable to water and dyes than other cells. Beans possessing the greatest number of these cells would swell more rapidly than others.

5. This greater permeability of these cells is due, in the first place, to a thinner cuticular or wax covering, or in the second place to one which is less efficient than in normal cells. Further, a more rapid absorption of water and swelling of the cell colloids takes place in these cells permitting of the more rapid passage of dyes through them. The second phenomenon may be a consequence of the first.

6. The ridges are more permeable to dyes than the hollows, due, firstly, to the removal of the waxy covering in that area and secondly because the coat is already stretched necessitating a smaller amount of water absorption to swell the colloids and open the palisade canals to permit of the passage of dyes, and consequently a shorter interval of time.

7. The more rapid penetrations of dyes through any

wrinkles that appear on the coat during soaking is from a similar cause. Bending of the coat causes a mechanical stretching of the tops of the palisade cells opening the spaces in the colloid and the palisade canals, allowing the dye to pass.

8. When the micropyle opens to permit of the entry of dyes, the acidic dyes enter and pass round underneath the coat in the nutrient layer and hour-glass cells. Basic dyes enter for a short way and then further penetration stops. The dye is held close behind the micropyle.

9. The holding of the dyes close to the micropyle is due to the formation by the basic dye with the tannin of a precipitate in the coat effectively blocking any further passage.

10. The rate of passage of the basic dyes through the coat is in the inverse ratio to the size of the molecule.

11. There is no lateral movement of dyes in the palisade cells and hour-glass cells during the passage of dyes through the coat.

ADDENDUM TO WORK WITH DYES.

As an expansion of the work on dyes the

effect of soaking beans in solutions of two iron salts, Ferrous and Ferric sulphate was tried. In the case of Ferrous sulphate, the salt penetrated directly through the coat, giving on the way a blue coloured precipitate with the tannin in the palisade, hour-glass and nutrient layer cells. The areas of greatest penetration were on the ridges and any raised portion of the coat. When the micropyle opened there was a slight penetration of the salt into the nutrient layer, giving a blue colour in the area, but owing to the precipitation of the salt causing a blockage, it did not penetrate far.

On the other hand, Ferric sulphate did not penetrate the coat at all except by way of cracks, but when the micropyle opened there was a slight penetration into the nutrient layer, giving a blue colouration and a bluish green thread in the hour-glass cells, further lateral spread being prevented by the paid of precipitate formed at the micropyle

Ferric chloride was found to behave in exactly the same way as Ferric sulphate, there being no penetration of the salt until the micropyle opened.

It is suggested that the difference in the mode of penetration of these three salts might be due to the effect of the ferric ion on the bean coat causing a coagulation of the seed-coat colloids. If this was so then the permeability of the coat to other

substances would also have been altered. Consequently the effect of offering a solution of .1% Ferric sulphate and .1% Basic Fuchsin, in equal proportions was tried. At the same time the effect of a solution of .1% Ferrous sulphate and .1% Basic Fuchsin was also tried. The beans were allowed to soak in these solutions at laboratory temperature for approximately twenty-four hours and then examined. Beans in the Ferric sulphate-fuchsin solution appeared as if they had been soaked in Fuchsin alone, except for a slight purplish patch close to the micropyle. Sections through the coat gave the normal red colour in the palisade hour-glass and nutrient layer cells. At the micropyle, sections through the purplish patch indicated that the colour was due to the presence of the blue precipitate formed by the tannin and the Ferric sulphate, plus the red colour of the Basic fuchsin.

The bean coats in the Ferrous sulphate-Fuchsin solution had a purplish red appearance, due to the penetration of both the Ferrous sulphate and the Basic fuchsin, the combination of the two colours giving the purplish tinge.

These tests showed that the Ferric ion had not coagulated the coat colloid as then the entry of the Basic Fuchsin would have been impeded or altered.

From the experiments with Ferrous sulphate

it would seem possible that although a substance is precipitated by the tannin in the coat yet that substance could still penetrate through the coat, the precipitate not preventing penetration to any great extent.

DISCUSSION AND CONCLUSION.

The expansion of the work on dyes using the iron salts can be explained on a similar basis to the dye work. The excess charge carried by the Ferric salt causes it to be adsorbed by the colloid of the coat, while the Ferrous salt is able to pass through as it is not so highly charged. Any crack in the coat however, allows both to pass in.

The experiments with the mixtures of dyes and salts indicated that two substances in solution could pass through the coat independently of each other and that they did not visibly affect each other's progress into the living tissues of the seed through the protoplasmic cell membrane.

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THE EFFECT OF SUBSTANCES IN SOLUTION IN THE EXTERNAL MEDIUM ON THE SWELLING RATE OF BEAN SEEDS.

The method adopted was a modification of that used by Shull (1920) in similar experiments. The beans were weighed dry and placed separately in numbered dishes, then each bean was wetted with the solution, removed immediately, dried with a piece of linen, and weighed again. This second figure gave the initial wet weight and was used as the reference point for all future weighings.

The dishes were placed in a thermostatically controlled incubator, running at 20°C, in order to maintain as constant an external atmosphere as possible. The beans were removed individually at two hourly intervals, and the superfluous moisture having been dried off with a well-washed linen rag, they were re-weighed and replaced in a fresh solution which was standing ready to be run into the dishes. This process of drying, weighing and replacing in fresh solution took about 1½-2 minutes per bean.

The first salt to be tested was Tolyl mercuri acetate. Beans with no cracks in the coat, and no other visible flaws, were carefully selected from the stock supply and placed singly in crystallising dishes, 1½ inches in diameter and 1

inch deep, which held plus the bean 25 cc. of solution. As the salt is extremely sparingly soluble a saturated solution was used, 2 grms. being added to a 1000 cc. of distilled water.

A control experiment was run at the same time, distilled water being used instead of the salt solution.

Any bean whose coat fractured during the experiment was discarded as the crack would at once allow the solute and solvent to reach the cotyledons without the intervention of the imperfect, semi-permeable membrane of the seed-coat. Any bean which cracked while soaking in the distilled water was also similarly discarded.

The weighings taken regularly at two hourly intervals during the twenty four hours, and also further weighings taken at irregular intervals up to 64 hours, are given in Tables I and II. The further weighings up to 64 hours were only recorded for the salt solution as it was obvious that the beans had not become fully swollen at the end of twenty-four hours.

At the end of twenty-four hours only ten of the beans soaked in the salt solutions had uncracked coats, and only nine in the distilled water control. The average of the weighings of these whole beans was taken, and the graph of the

increase in weight of beans soaked in salt solution and the graph of the increase in weight of beans soaked in distilled water were drawn. By a comparison of these two graphs it can be seen that the one for the increase in weight in distilled water shows a much steeper rise than that for the salt solution.

Exactly similar experiments were carried out with two further salts, Mercurated o-chlorophenol and Mercurated o-nitrophenol. Saturated solutions of both these salts were prepared (1·5 gms. of salt being dissolved in a 1000 cc. of distilled water in both cases). The weighings of these tests were taken at two hourly intervals up to thirty hours. As before, control experiments were run at the same time and under the same conditions, using distilled water as the external medium. The weighings taken during these experiments are given in Tables III, IV, V and VI. As in the previous experiment any beans whose coats cracked during the period were discarded, and the average of the weighings of the beans with uncracked coats was taken and graphs were drawn.

On comparison of all the graphs from these two experiments it can be seen that the slope of the increase in weight of the beans in distilled water was almost the same as the slope of the increase of

the weight in salt solutions, except for a slightly slower, and lower, rise in the solution of Mercurated o-chlorophenol. This is probably due to the solution not giving up water quite as readily as the distilled water, but the graph has the same general shape as that for distilled water.

DISCUSSION.

From these experiments it would appear that there is a marked difference in the behaviour of beans soaked in the first salt, Tolyl mercuri acetate, as against beans soaked in solutions of Mercurated o-chlorophenol and Mercurated o-nitrophenol.

After a consideration of the work on dyes it was apparent that the salts would enter the seed in either of two ways:- (1) Mainly through the coat and slightly through the micropyle. (2) Slightly through the coat and mainly through the micropyle depending on the charge carried by the salt in solution. Thus the salt could affect the intake of the water in two ways, quite apart from any slowing up due to the osmotic pressure of the solution, namely by slowing up the intake of water through the coat or by slowing up the intake of water through the micropyle. Shull (1913) showed that the initial intake of water from a solution had almost as great

an initial rapidity as when pure water is used until the osmotic pressure of the salt on the outside balances the internal forces, then the entry of the water ceases. But the osmotic pressure of the solutions used in this case are too low to account for the discrepancy between the salts.

An attempt was made to trace the path of entry of the salts by placing sections of the bean coat (taken from beans soaked in the various solutions) in Deniges Mercury indicator solution. No visible precipitates were obtained in any of the sections and their non-appearance rendered this method useless for the purpose, although other investigators working with different seeds have claimed to be able to trace the path of entry by such a method.

Returning to the dye work it was noted that basic dyes which entered through the coat produced a precipitate with a coat extract solution while the acidic dyes did not.

A solution of coat extract was made in exactly the same way as in the dye experiments, and was used as a test solution. A white precipitate was obtained with the solution of Tolyl mercuri acetate, but not with the solutions of Mercurated o-chlorophenol and Mercurated o-nitrophenol. Previous removal of the pyrogallol tannin by precipitation with gelatin

considerably reduced the amount of the precipitate obtained (to nil in most cases). This would indicate (as with basic dyes) that it was the tannin which was mainly responsible for the precipitate. Either the salt coagulated the tannin, which would block the pores of the coat, and the micropyle, or else there was a direct combination of salt and tannin.

It is postulated that by analogy the first salt will enter by way of the coat and micropyle, while the latter two will enter mainly through the micropyle. The precipitate produced by the coat-extract solution (which is mainly pyrogallol tannin) with the first salt will block the pores of the coat, preventing the easy ingress of water. This would account to a certain extent for a discrepancy. The main difference, however, is due to the precipitate blocking up the micropyle as soon as it opened, and preventing it from playing any effective part in water absorption. A plug of white precipitate is visible in the micropyle of beans soaked in this salt solution, and there is also a small mass of the salt just within the micropyle.

Contributing to the discrepancy noted, was the fact that these beans had not long been harvested and were not properly dry; in such beans the micropyle becomes effective in the intake of water more rapidly than in drier beans. Consequently anything interfering

with the micropyle mechanism for water absorption would have a much greater effect on these beans than on those stored for a longer period.

CONCLUSIONS.

The discrepancy between the behaviour of the beans soaked in the solution of Tolyl mercuri acetate and that of the beans soaked in the solutions of Mercurated o-chlorophenol and Mercurated o-nitrophenol is due partly to the blocking of the semi-permeable membrane of the seed-coat by the precipitate formed by the salt with the coat extract solution. The large size of the molecule renders the blockage more effective than is the case with the smaller iron and basic dye molecule in which the blockage is not moticeable. The main cause of the discrepancy is no doubt due to the blocking of the micropyle by a plug of this same precipitate, preventing the rapid ingress of water by this path; the unripe state of the beans being a contributing factor. It is extremely probably that any other salt with a large molecule, giving a marked precipitate with the seed-coat extract, would behave in a similar manner and cut down the rate of water intake.

LITERATURE.

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EFFECT ON GERMINATION OF SOAKING BEANS IN SATURATED SOLUTIONS OF THE VARIOUS SALTS.

A saturated solution of Tolyl mercuri acetate was made by adding 2 grms. of the salt to 1000 cc. of distilled water. This solution was allowed to stand overnight and was used on the following morning. Beans, which had been selected as being free from any visible flaws, were placed in groups of twenty or twenty-five in petri-dishes, enough solution being added to cover the beans completely, and the dishes were left on the bench at normal laboratory temperature. The actual temperature of the solution in the petri-dishes was taken at the beginning and at the end of the periods of soaking, i.e. after 48 hours, and in no case was there a variation in temperature greater than 2°C. At definite intervals of time the superfluous solution was poured off from the dish, and all the non-cracked beans from that dish were sown, with hilum uppermost, in clean sand. Petri-dishes were used in order to allow of the complete aeration of the solutions during the whole time, and to permit of each bean being at the same depth in the solution, also preventing the presence of excess water or solution which, as has been shown by Kidd and West (1919), is harmful to subsequent germination and development.

At the same time, controls were run with beans soaked in distilled water. The beans, from the distilled water, were sown in alternate rows with those that had been soaked in the salt solution. As a further control of the effect of soaking pure and simple, untreated dry beans were also sown at the same time-intervals as were used for the soaked beans.

Every morning the sand layer covering the beans was removed and the number of germinations (breaking of the seed coat by the radicle) counted. As soon as the radicle had appeared the bean was inserted in its normal position in the sand, i.e. root downwards. The number of germinations per day were noted, and the results are given in Tables VII, VIII and IX. The total number of germinations has been calculated as a percentage of the total number of beans sown in that particular test. The sowing date for the 24 hour, and over, periods of soaking was taken as the day soaking started.

From a study of the figures given in the tables there is an indication that there may be a slight increase in rapidity of germination in those beans soaked in salt solution for half an hour. After 8 hours soaking in the solution there seems to be a marked retardation of germination, and beans soaked for 48 hours are completely inhibited from germinating. The salt would also appear to prevent the beneficial

effect of soaking in the four to eight hour period, due possibly to the slower swelling rate of the beans in the salt solution retarding germination.

The beans which, having been soaked in salt solution, failed to germinate were not without interest. They differed markedly in appearance from beans soaked in distilled water. The ungerminated beans from the salt solution appeared greasy to the touch, were yellowish brown in colour, and were much more swollen than the beans from the distilled water, which had a clear light yellow colour. This change in colour appeared to be due to an alteration in the colour of the cells of the coat, as was shown by sections, and following a pathological change brought about by the death of the bean owing to the salt. The increase in size of the bean may be attributed to an abnormal intake of water, due to the release of a large quantity of osmotic substances as a result of the death of the bean tissues, or may be explained by an alteration of the solvation characteristics of the testa colloids.

In some beans, soaked in the salt solution, where germination did not take place the root was curved, twisted, stumpy and short (Fig.11). This curvature was examined, and in sections it could be seen that the cells on the concave side of the root were dead while those on the convex side continued growth (Fig.12). This would naturally cause curvature of the root, forming a concavity on the side bearing

the damaged cells. Apparently only a small area on the upper surface of the root, lying closest to the surface of the bean coat, was injured and prevented from developing, the other tissues which lie deeper beneath the surface being uninjured. The plumule in these beans appeared to be perfectly normal. This lack of effect on the plumule is probably due to its having been protected from contact with the solution by reason of its position between the cotyledons, and by its greater depth beneath the coat. On the other hand the root tip, lying much nearer the surface and therefore more exposed to the salt, showed injury, and it is noteworthy that the injury occurred on the side most exposed to the salt solution.

It is suggested that the salt enters through the coat in the first instance, and while it may not reach the embryo during the actual period of soaking it does so while the bean is lying in the sand and absorbing more water, and then causes damage to the upper surface of the radicle. The amount of salt absorbed into the coat during soaking is therefore the important factor to be considered. While some of the salt must be precipitated in its passage through the coat and might be injurious, on the other hand sufficient non-precipitated salt may be left to bring about the root curvature.

Zimmerman and Crocker (1934) discovered

that plants were susceptible to injury from vapours emanating from soil moistened with bichloride of mercury solution. The injuries were similar to those induced by metallic mercury vapour. They postulated that the mercurial salts were being reduced by the organic matter in the soil, and that free mercury vapour was being released into the atmosphere, causing subsequent injury to the plants. It is suggested that a similar phenomenon may be taking place in the bean. The tannin in the coat, acting as a reducing agent, might release free mercury vapour which would injure the root and bring about the curvature of this organ, the mercury here being the injurious agent and not the salt itself. It does not seem very probable that this reduction of the salt is actually taking place, but it is a possibility nevertheless.

Beans which had been soaked for longer intervals of time did not germinate. In these cases the micropyle probably opened and allowed the salt to enter. Apparently some of the salt must have reached the actual tip and the under surface of the root, causing the death of these tissues, in addition to the death of the upper surface tissues caused by the salt which had penetrated the coat. This would cause the death of the whole radicle, and if the salt penetrated further, by way of the coat and micropyle,

the plumule would die also. In other cases the plumule had developed although the root was badly injured, or sometimes completely destroyed. Protection in the first instance would thus appear to depend on the depth of the coat covering the tissue, and, secondly, on the length of the path the salt has to transverse from the micropyle to the embryonic tissue.

As stated previously, a slight increase in the rapidity of germination was noted in beans soaked for half an hour in salt solution. A second test, identical with the first, was made to check this, the length of time of soaking being shorter. The results of this test are given in Tables X, XI and XII. These results, however, do not confirm the suggestion that a short period of soaking in salt solution expedites germination.

A further test was carried out on lines identical with the first two tests, but in this case the beans were sown root downwards. The beans were left undisturbed in the sand and were watered each morning with distilled water, every endeavour being made to keep the beans covered with a uniform depth of sand. The appearance of the plumule above the surface of the sand was taken as the first sign of germination, as this would be a better criterion of the actual effects of soaking, for in some cases the

root may burst the coat and growth would cease without the plumule developing at all, vide Hicks (1900) and Atwood (1922).

From the results of this experiment, as given in Tables XIII, XIV and XV, it would appear that soaking for two hours was the most that the beans could be subjected to without causing injury, subsequently, to the seedling plant. In that time the salt had either penetrated through the coat, or else sufficient had been absorbed into the coat to allow of its reaching the root tissues during the further swelling of the beans in the sand. The roots of some of the beans had the same curved, short and stumpy appearance as before, but a further development had taken place and a thin, whip-like primary root had been produced from the stumpy root, possibly by a form of regeneration from the uninjured and more deeply placed root tissues. It is probable that such plants would have been able to establish themselves in the soil, but growth and development would most certainly have been retarded, and the next generation might have been affected.

It sometimes happens that a salt which acts as a poison in comparatively concentrated solutions will act as a stimulant in more dilute solutions. Further germination tests were therefore embarked upon using more dilute solutions. The method of

procedure was similar to that adopted in the previous experiment, only, in this case, the supernatant, saturated fluid was decanted off and was added to an equal quantity of distilled water, thus giving a concentration equal to half that previously used. A comparison between the beans soaked in this half-strength solution and those soaked in distilled water was then made, and the results of this are embodied in Tables XVI and XVII, and the type of seedling produced is given in Table XVIII.

In these tests the effect of the salt on germination alone does not become apparent until after soaking for eight hours (Fig.13), but when the class of seedling developed is examined it is seen at once that some of the seedlings would not be able to establish themselves as healthy plants.

From this experiment it would appear that dilution of the salt has little or no effect on its poisonous properties. There is a slight increase in the germination power of beans soaked for longer periods, but this may have been due to changes in the beans themselves, resulting from longer storage prior to experimentation rather than from the dilution of the salt solution.

An attempt was made to discover the relationship between the increase in weight of a bean soaked in the salt solution and its power of

germination. The same relationship for beans soaked in distilled water being used as a control.

The method adopted for these tests was very similar to that employed for discovering the effect of saturated solutions on the swelling rate. In this case, however, the selected beans were soaked singly in crystallising dishes in solutions unchanged throughout the experiment. The dry weight was taken before the commencement of the experiment, and the final wet weight before the beans were sown. The germination dates were noted, and the type of seedling plant produced was recorded, the results of the experiment being given in table XIX. Examination of this table discloses the fact that damage is caused to any bean which has absorbed from the solution sufficient to give an increase of weight equal to approximately 10% of its dry weight.

The results of the distilled water experiment are given in Table XX. Any correlation between the absorption rate and germination rate is difficult to find, but, within limits, it would appear that the greatest swelling rate gave the most rapid germination.

Some further tests were carried out on similar lines, the number of hours of soaking, however, being reduced to six. Results of this experiment are given in Table XXI. One fact that

stands out clearly in this Table is that beans which develop cracks in the coat during soaking produce abnormal seedlings. This means most certainly that any bean which was cracked prior to soaking would be injured by the salt, and probably destroyed, as the salt can reach the young embryo directly.

A photograph of beans 13, 7, 9 and 5 (Fig.14) shows the effect of the salt on the bean root: number 13 is the most affected and yet it has not the greatest percentage increase in weight. It is suggested that perhaps the position of the area of wrinkling of the coat during soaking might throw more light on this matter. If the area of wrinkling and thus the area of greatest permeability, should occur immediately above the embryo, then more damage would result than if the wrinkling occurred in some other area. In order to test the validity of this supposition a further test was carried out, in this case the area of the bean in which wrinkling occurred during soaking being noted, as well as the increase in weight and other facts. The results of this experiment are given in Table XXII.

From this Table it will be seen that the most dangerous area in which wrinkling may occur is that towards the top of the hilum and over the embryo. Beans which showed wrinkling in this area also showed injury in the resultant seedling. This would indicate

that the supposition that salt poisoning, at least in its early stages, takes place through the wrinkled coat to the most exposed part of the embryo root, namely near the base, is correct. This injury causes curvature of the root, but if the wrinkling has proceeded far enough, or is so situated that the salt is allowed to reach the embryonic root tip, then death of that tissue will ensue.

The control experiment, using distilled water instead of salt solution, did not indicate much, judging by the results as shown in Table XXIII, except to suggest that rapidity of germination follows rapidity of swelling fairly closely.

In order to test the effect of the salt on the subsequent development of the plant (apart from its effect on germination) a series of experiments were conducted. Beans were soaked for twelve hours in a saturated solution of Tolyl mercuri acetate at laboratory temperature. After soaking the beans were sown in soil in 5 inch pots which were placed in a greenhouse. The pots were watered every morning and the number of germinated beans noted, and, after germination, the general morphological appearance of the seedlings produced was recorded. The results of the experiment are given in Table XXIV.

This Table indicates that the salt has slowed down the germination rate. This "slowing down"

is also apparent in the subsequent development and growth of the plants produced from beans soaked in the salt solution. These plants had a slower rate of growth and were smaller in stature than those from beans soaked in distilled water, or from beans that were untreated. This "slowing down" of the development also influenced the date of flowering of the various classes of bean plants. Plants derived from untreated beans, and from those soaked in distilled water, opened earlier (28th May) than those from the beans soaked in salt solution (5th June). This slowness of development and retardation of the growth rate is probably directly related to the injury of the root caused by the salt. The root system will take longer to develop, and as a consequence the plant will take longer to establish itself. Further, the root system of plants from treated seed will be decreased, thus reducing the area for absorption of water and salts from the soil, with consequent repercussion on the aerial parts of the plant.

Experiments were now conducted with a view to discovering if the poisoning effect of Tolyl mercuri acetate could not be prevented in some manner or other, in order that fungi infecting the surface of the seed coat only could be destroyed while the bean itself remained undamaged.

Arising out of the work on the localisation of the penetration of dyes by means of a protective paraffin-wax covering, efforts were now made to discover that if by waxing the bean coat the embryo could be protected from the poisoning effect of the salt.

Attempts were first made to apply the wax to the area of the coat immediately about the embryo itself, and, using a short period of soaking, to discover if the embryo had been protected from injury. Unfortunately, in the majority of cases, the swelling of the coat caused the wax to be thrown off, thus exposing the area it was desired to protect. Nevertheless, some measure of protection appeared to be afforded by the experiment as beans which retained the wax produced normal seedlings. These facts are inconclusive, however, since such beans might have produced normal seedlings without the wax covering, because the very fact that the wax remained intact makes it obvious that swelling and wrinkling of the coat did not occur in the region of the embryo in these particular beans, the absence of swelling being due to the constitution of the coat near that area, rendering it less permeable to water than other areas of the coat. It is reasonable to assume, however, that some measure of protection was afforded by the wax. Other protective agents were tried, but these were always sloughed off, or failed to adhere to the

coat on account of its own waxy covering.

A further series of experiments, again using paraffin-wax as a protective agent, was conducted, in this case not with a view to preventing injury to the embryo in the early stages of soaking, but in order to prevent or minimise injury in the longer periods of soaking, over twenty-four and forty-eight hours.

The method adopted was to select beans free from cracks and visible flaws, and dip them in hot paraffin-wax (52°C. M.P.). This coating was allowed to harden. The area covered in this way included the hilum, micropyle and part of the hilar bulges (Fig.15). These beans were placed in petri-dishes, and were soaked for 24 and 48 hour periods in a saturated solution of salt. They were then shaken free from superfluous solution, sown in damp sand, and watered each day, the appearance of the root tip being taken as the first sign of germination. Control experiments, using unwaxed beans, were also conducted. The results of the experiment are given in Table XXV.

The percentage germination of the waxed beans shows a marked improvement over those beans which had not been waxed - 88.3% and 75.0% as against 45.0% and 0% for the 24 hour and 48 hour periods. In some cases the wax had become separated from the coat during the soaking, and although it had not actually

come off it is doubtful if in this condition it would prevent the access of the salt to the micropyle; however it would at least hinder its passage considerably and reduce the poisoning effect.

A series of experiments on lines somewhat similar to those just mentioned was then undertaken, in which different parts of the hilar area were waxed, the hot wax being applied by means of a seeker to the hilum and micropyle in some beans, and to the micropyle and a certain small area of the surrounding coat surface in others. As before control experiments were set up, using unwaxed beans. The period of soaking was 24 hours in these cases. The results are embodied in Table XXVI.

Again there was an improvement in germination percentage of the waxed beans as compared with unwaxed beans - 72.8% when the hilum and micropyle were waxed, and 85.3% when the micropyle alone was waxed, as against 31.6% in the case of the unwaxed beans. It will be noted that there is a third class of beans included in the Table - those which had lost their protective covering during the soaking. In other cases it is doubtful if the wax really prevented the intake of water or salts by the micropyle. As before, other protective solutions were tried but with no greater measure of success.

In the previous experiments the appearance

of the radicle at the surface of the sand alone was noted, and the subsequent development of the seedling was not considered. It was thought advisable in consequence to conduct an experiment during the course of which the character of the seedling would be observed. In a further sample of beans the micropyle area was waxed as it was realised that this was the danger point. The time of soaking for this experiment was 24 hours, and the results are given in Table XXVII.

The germination figure is seen to be 93.6%, which is higher than that obtained for unwaxed beans soaked for 24 hours. From this, and judging by the class of seedling produced, it is obvious that waxing of the micropyle acted as a protection to a certain degree. Other factors, however, must come into play, otherwise the class of seedling produced would have been uniform.

As wrinkling increases permeability the locus where this occurs conditions the degree of damage done to the embryo. Wrinkling directly over the axis of the embryo results in immediate damage, while wrinkling over the cotyledons may cause little or no damage. It is possible also that the wax seal was not perfect and that the salt gained a passage through the micropyle and attacked the root.

At an earlier stage in the course of the experimental work it was stated that the poisoning

of the root might be caused by a penetration of the salt through the coat, or by way of the micropyle, during the period when the bean was lying in the damp sand prior to germination, and not during the actual period of soaking. Thus any salt which was clinging to the coat after soaking might in this way reach the bean, or any salt clinging to the coat might poison the root as it passed through the coat at germination. The removal of this excess salt on the coat might help to reduce the injuries to the seedlings. To test the validity of the hypothesis experiments were conducted in which beans, after having been soaked in the solution, were washed for about five minutes in running water and then sown in damp sand. Control experiments were conducted with beans which were not washed after soaking. It was considered of further interest to compare beans which, besides being washed at the end of the period of soaking, had had the micropyle waxed for the whole period. The results of these experiments are given in Tables XXVIII and XXIX. The class of seedling produced was not noted for the shorter period of soaking.

From these results it would appear that washing the beans after soaking does improve the percentage of germination obtained. This is probably due not so much to the removal of the superfluous salt from the actual surface of the coat, but rather

to the removal of the excess salt in the micropyle area and particularly the plug blocking the micropyle. If this plug is left in the micropyle, as the bean gradually takes up more water from the sand prior to germination some of the salt from this plug may move in through the micropyle and reach the embryonic root, causing injury or death. That this is the most probable explanation of the benefit accruing from washing can be proved by a study of the beans which had the micropyle waxed for the whole period of the experiment. In this group the percentage of germination is very much higher although the seedlings are by no means normal, yet these figures do indicate that it is the passage way through the micropyle that is the danger point. Washing of the beans soaked for short periods does appear therefore to afford some measure of protection to the seedling.

Another method of protection, entirely different from the previous method of waxing, was now embarked upon. It has been demonstrated that certain ions antagonise the passage of other ions through a membrane and it was decided to try the effect of mixing Tolyl mercuri acetate with certain inorganic salts and testing the effect of these solutions on germination. A control experiment with only the salt in solution was run at the same time. The inorganic salts used were Sodium sulphate, Ferrous sulphate and Calcium sulphate. The results are given

in Tables XXX, XXI, XXXII and XXXIII, and the class of seedlings produced in Table XXIV.

The protective effect, if any, afforded by these salts is not sufficiently marked to warrant further consideration, although according to the theory the Sodium ion should increase the permeability of the coat, while the others should decrease it, and in fact this is borne out by the results of the experiment, the Sodium mixture causing the greatest injury and the others less.

As an expansion of this work a solution of coat extract was used to mix with the salt solution instead of the inorganic salts, and the effect of the resulting mixture on germination tested. A definite precipitate was produced when the two solutions were mixed, and this was filtered off in one solution and left in the other. An excess amount of coat extract solution was added to ensure complete precipitation of the salt. In the control experiment the same amount of distilled water was added to the salt solution to render it of the same strength as the coat extract plus salt solution.

These solutions were actually used in the different groups of beans:-

(a) 10 cc saturated salt solution plus 50 cc distilled water.

(b) " " " " " " 50 cc coat extract solution.

(c) " " " " " " 50 cc coat extract solution

filtered free of precipitate. All the beans were soaked for the same period - 7 hours 45 minutes - in the normal way. The results of the experiment are given in Table XXXV and the class of seedling in Table XXXVI.

From these tables it can easily be seen that the seed-coat extract has some considerable effect on the salt solution, reducing its poisonous properties considerably (see Figures which show the differential effect of the various solutions). The filtered solution appears to be less poisonous than the non-filtered solution, probably due to the fact that some of the salt was not precipitated, although excess of coat extract solution was added. The non-precipitated salt is caught in the precipitate and removed when filtered, but is left in the unfiltered solution and can still cause damage.

The fact that the coat extract solution removed the salt from the field of activity was considered to be due to the pyrogallol tannin forming a precipitate with the salt, as shown earlier in the experimental work. This is of interest as the tannin appears to oxidise as the bean matures, and it may precipitate more or less salt as it oxidises or otherwise changes as the coat darkens in colour, thus affording more or less protection to the bean. Similarly the presence of more or less tannin in

individual beans will alter the amount of protection they receive. At present the indication is that older beans are less liable to poisoning than younger beans, but this may not be due to the tannin, but rather to hysteresis of the coat colloids.

At one time it was considered possible that the mixing of the salt and the coat extract solutions produced not a precipitate but a salting out of the tannin, the salt being absorbed on to this salted-out precipitate. But the fact that the other salts did not produce the same salting out led to the belief that there is actually some combination between the tannin and the salt.

In order to test the efficiency of the precipitating mechanism, beans from which the testa had been carefully removed were soaked in the same three solutions as before, with a control in distilled water. The beans soaked in any of the solutions containing salt did not germinate, but those in distilled water did. Whether total precipitation of the salt was not obtained, or else the precipitation mechanism is imperfect, is not definitely known, but it seems most likely that incomplete precipitation of the salt was the cause of non-germination.

In order to discover if it is the mercury ion of the salt which is toxic to the bean, the effect on germination of soaking beans in saturated solutions

of various other mercury salts was tested. The experiments were conducted on exactly similar lines to those used in testing Tolyl mercuri acetate. The effect of Mercurated o-chlorophenol was tried first. As before, groups of twenty beans were soaked (in petri-dishes) in a solution containing two grams per thousand cc. at laboratory temperature. The results are given in Tables XXXVII and XXXVIII, the appearance of the root tip being taken as the first sign of germination.

In this test there appeared to be little or no difference between the effect of the salt solution and the distilled water on the germination rate, but there is a slight increase in the rate of both as compared with the untreated beans.

A further test was made, and in this case the appearance of the plumule at or above soil level was taken as the first sign of germination. This was done as it was considered possible that the radicle might just burst the coat and then develop no further, giving a false measure of germination in the first test. The type of seedling produced was also noted. The results of this experiment are given in Tables XXXIX, XL and XLI.

The effect of this salt on germination, and the class of seedling produced, would not appear, from a study of the tables, to be very different from the effect obtained by using distilled water. There

is the same slight increase in the rate of germination over that of untreated beans - due to the beneficial effect of soaking. It would appear then that the mere presence of the Mercury ion in a salt does not necessarily render it poisonous. Even if this salt did not penetrate the coat in the first instance, but could only enter the coat when fully saturated, or through the micropyle, then the toxic action, if any, should have been apparent in the beans soaked for a longer period.

The same treatment was applied to beans when testing the effect of Mercurated o-nitrophenol on germination. The results are given in Tables XLII and XLIII, the appearance of the root being taken as the first sign of germination. The beans were then inverted and the plants grown on. The rate of germination, and the type of plant produced, were both similar to that in distilled water, and this salt, as in the case of the previous salt, would appear to be inoxious.

The effect of Shirlan on germination was tested on the same lines as before, only in this case no previous test - using the root as the first sign of germination - was conducted. The appearance of the plumule in beans which had been soaked in a saturated solution of the salt was noted and also the type of seedling produced. The class of untreated

beans was discarded in this test also, as the actual comparison made is between the effect of the salt and the effect of distilled water. The results of this experiment are given in Tables XLIV, XLV and XLVI.

The effect of this salt on germination is not different from that of distilled water to any marked extent, and the seedling plants were mostly normal, any abnormalities not being attributed to the action of the salt.

The remaining salt, Sodium - 2 - (hydroxy-mercuri) benzoate, was employed as before, and the results of the experiment are given in Tables XLVII, XLVIII and XLIX.

The action of this salt on the beans recalls that produced by Tolyl mercuri acetate, but in a much milder form. Soaking up to eight hours has no injurious effects, but in the longer periods of soaking there appears to be a slowing up of germination and a poisoning of the root. The fact that poisoning does not occur, except with the longer periods of soaking, might be due to several causes. Either the salt is less toxic, and larger quantities are necessary to cause poisoning, or else the salt is precipitated more completely through the coat, necessitating a longer period before the salt could reach the embryo. There is a third possibility that this salt might behave like the acidic dyes and enter

by way of the micropyle only. This would explain the fact that no poisoning occurred before eight hours as the micropyle is not very effective before that period has elapsed. This third theory, although attractive, does not appear likely as this salt behaves like the basic dyes, producing a precipitate with a coat extract solution.

DISCUSSION.

It is now possible to discuss the effects of these various salts on germination and their methods of penetrating the coat. Previous to and during the course of the present work other investigators had been experimenting with the effect of water and salt solutions on germination, and the results obtained are extremely conflicting.

Many workers had stated previously that soaking seeds, such as those of Phaseolus species and others, in water alone reduced germination and produced poorer plants, but so far as the author is aware none of these statements were applicable to Vicia Faba, which according to Kidd and West (1917), can withstand soaking up to 72 hours. Consequently any injury to the bean caused by soaking in a salt solution could fairly be attributed to the salt and not to any harmful effect of soaking alone.

There is no doubt from a study of the

experimental work carried out with Tolyl mercuri acetate that there is a marked retarding effect on germination and injury to the subsequent seedling. The injury in the first place would appear to be due to the passage of the salt through the coat reaching the base or crown of the root. This passage through the coat is most marked in those beans in which wrinkling of the coat occurs directly above the embryo - the coat being more permeable to substances when wrinkled. The presence of more, or fewer, of these cells or groups of cells, which are more permeable than the average cell in this area just above the embryo, would account for the individuality displayed by the beans, why some were poisoned and some were not. Wrinkling in any other area did not cause the slightest damage to the bean. In the later stages penetration would take place all over the coat and also through the micropyle. This is similar to the findings of Port (1932). The salt which penetrated the micropyle caused total death of the root tissue as it came in contact with the young growing tip. In the beans soaked for longer periods the coat would be totally permeable, consequently the whole root tissue would be killed, although in some cases the plumule, lying deeper still beneath the coat and further from the micropyle, would not be damaged and could develop.

Those beans which developed thin whip-like roots, due to the poisoning of the outer cells of the root, resemble the injured peas described by Brenchley (1927), this type of injury being described as "strangulation". This injury to the root caused by the salt is without doubt the main reason for the slow development of plants produced from treated seed. The efficiency of the root is diminished and thus the efficiency of the plant is lowered also. This would render the use of this particular salt dangerous in practice as the value of the crop produced would be considerably reduced.

It is generally understood that a substance which is toxic in high concentrations is often stimulating in lower concentrations. This was not found to be the case with this particular salt. No doubt dilution would render the salt less harmful and finally a critical point would be reached at which no injury would be done to the bean, no matter how much the soaking was prolonged.

The brown and swollen appearance of the dead beans soaked in salt, after they had been in sand for some considerable time, indicates that, as suggested by Shull (1932), the poisoning by the salt has led to the dissolution of the embryonic tissues, and this has given rise to a liquid with a high osmotic pressure, causing a large amount of water to

be absorbed and giving a heavy and swollen seed.

Ths use of wax as a protective covering was based on the discovery that there is no lateral movement of a substance while passing through the coat. Thus if the area covering the embryo could be rendered totally impermeable to a poisonous salt, protection would be afforded to the embryo in the early stages of soaking, or, at least, until the micropyle became effective and allowed the salt to pass through. This fact, that there is no lateral movement of a substance passing through the seed-coat, is supported by Shull (1930) and by Braun (1924). On the other hand, Collins (1918) and Harrington and Crocker (1923) maintained that there was a lateral movement of iodine in the sub-aleurone layer in the "seeds" of Barley and Grass. This lateral movement was only found when the "micropyle" had become effective thus permitting salts to pass through, but, from the findings given here, it is apparent that substances pass directly through the coat from the surface inwards, and do not move laterally while so doing. The difficulty experienced in keeping the wax covering in position rendered the results of doubtful value, but the increase in the number of normal seedlings produced indicated that a certain measure of protection had been afforded by the wax.

The further experiments with wax as a

protective agent covering the micropyle showed that in the later stages of swelling it is the micropyle that allows the salt to reach the root and injure it. While blocking of the micropyle did not prevent injury completely it resulted in a higher germination, and though the rate of germination was slower this is not attributed to the effect of the salt but rather to the decrease in water intake.

The next group of experiments was conducted with a view to discovering if the hypothesis that the salt would lie on the coat and penetrate to the embryo while lying in the damp sand is correct, as Niethammer (1928) suggested was the case in cereals. The removal of the excess salt from the surface of the bean would prevent any penetration of salt into the bean from outside during the period it was lying on the sand. In this way the injury to the bean should be reduced, although any salt already in the coat could still pass through and damage the bean as more water was absorbed and the intermicellar spaces and palisade canals opened. No doubt some of the salt was also mechanically and electrically adsorbed by the surface of the coat and was not removed by washing. The percentage of germination was improved, and, as suggested, it is possible that further penetration of the coat does take place in the seed bed. During the washing of the bean any salt clinging to the coat in

the micropyle area was also removed, and this would prevent any penetration of salt through the micropyle while the bean is in the sand, giving a bigger yield and a better class of seedling. This second hypothesis would be the most important in the case of seeds which were soaked until the micropyle had become effective. The plug of salt, always found in the micropyle, would be removed, and thus the source of poison would be eliminated. The statement is strengthened by the fact that beans examined microscopically after lying in sand did not possess a salt plug. The salt had been absorbed through the micropyle into the bean causing damage to the embryo. It would appear to be advantageous, therefore, to wash all beans which have been soaked in the salt solution before placing them in the sand.

It is a generally accepted hypothesis that ions of different nature presented to a membrane can interfere with one another, one affecting the penetration power of the other. Bivalent or tribalent ions tend to bring the particles of a membrane more closely together, closing up the intermicellar spaces, while monovalent ions have the opposite effect, hence the "antagonism" between ions of these two classes. Organic compounds are similarly affected by inorganic salts (Schreiner and Reed, 1908).

It is also generally agreed that the presence

of a second substance dissolved in the medium external to living tissue may reduce the harmful action of any toxic substance already present.

These theories were utilised in the series of experiments in which inorganic salts were mixed with Tolyl mercuri acetate, and the effect of the resulting solution on germination tested. The results followed the theoretical hypothesis closely, the bivalent ions protecting the bean embryo to a certain degree, while the monovalent ion rendered the poison more virulent. The actual seat of the "antagonism" may not be in the coat, but in the plasma membrane of the cells of the embryo.

The expansion of this work, using the seed coat extract instead of the inorganic salt, raises some points of interest. The precipitate formed by the salt and the coat extract solution removes a certain amount of the salt from the field of activity, reducing its poisonous properties. The filtering off of the precipitate further reduces the toxicity of the solution by the removal of the salt which may have absorbed on to the precipitate, but not actually combined with the tannin. This loosely held salt, if released, would be free to pass through the coat when the beans are placed in the unfiltered solution. This would account for the greater toxicity of the non-filtered solution. It is possible that not much

of the salt actually enters into direct combination with the tannin to produce the precipitate, but the salt may have a coagulating action on the tannin. The coagulated clots so formed would absorb salt on to the surface, and this would then remove it from the field of activity if the solution were subsequently filtered. It would require a huge amount of tannin solution to remove the salt completely from the field of activity by direct combination of the salt and tannin if the latter hypothesis was the case. That the hypothesis is likely is seen by the results obtained with beans which had their coats removed, and also from the fact that beans soaked in the coat extract salt solutions were poisoned.

The fact that a "precipitate" is produced with the tannin is of interest since Denny (1917) showed that a seed coat containing tannin was less permeable to water than one without tannin. Now it appears that tannin also prevents the entry of other substances to a certain extent. As this tannin appears to change its constitution as the beans age it is evident that older beans would be either more, or less, susceptible to the toxic action of the salt. At present the indication is that they are less susceptible. It is obvious that this precipitation or coagulation of the tannin of the coat must block the pores of the colloid and the palisade canals for

some time. Yet later as the pores widen, more salt enters and passes through the coat and poisons the embryo, or else salt which is already in the coat, and whose path is temporarily blocked by the precipitate, is later enabled to enter as the palisade canals widen. This is contrary to the findings of Harrington and Crocker (1923), who discovered that even the highly toxic salts could penetrate the "seed coat" of Johnson Grass only in exceedingly small subtoxic or slightly toxic doses, and stimulate it into growth.

Tests conducted with other salts showed that Mercurated o-chlorophenol, Mercurated o-nitrophenol and Shirlan were quite harmless to the beans, while Sodium-2-(hydroxymercuri) benzoate was toxic to a slight degree, the effect being reminiscent of Tolyl mercuri acetate. The poisoning would not appear to be due to the cation mercury alone, for if this were so the salts would have been toxic; unless some of the salts did not reach the root. Brown (1909) stated that freely dissociated mercuric salts could not pass through the seed coat of Hordeum vulgare, while slightly dissociated substances could pass. This theory might have been utilised to explain why some of the mercury salts were poisonous and others were not, but the fact that the micropyle would have opened during the longer periods of

soaking, and would have allowed any one of the salts to reach the root tissues, poisoning them if it was toxic, refutes this explanation. Thus it would appear that in the case of mercury salts the cation alone is not the toxic agent, as was attributed to the copper ion of copper salts by Breal and Giustiniani (1904).

Some authorities, e.g. Senf (1925), maintain that a stimulating effect is obtained with commercial fungicides, which often consist in large part of these organic mercury compounds. Examination of the results obtained by Senf and other workers show that in most cases the increase in germination gained was due to diseased seed having been used. The increase in germination, in comparison with the controls, being due to the fungicidal properties of the substances, and not to their stimulating action. Remy and Vasters (1923), and Becker (1926), carrying out similar experiments with chlorophenol quicksilver, attributed the increase in germination rate obtained by them to very diseased seed, the germination of healthy seed being unaltered by the salt. Up to the present it would appear that there is no actual evidence for any stimulating effect due to treatment of seeds with organic mercury salts. Certainly none was found during this investigation. The only increase in germination rate being caused by the

soaking of the seed and not by the salt.

The poisoning effect obtained with Tolyl mercuri acetate and with Sodium-2-(hydroxymercuri) benzoate is similar to that obtained by several workers, notably Czarnowski (1924-1925), who was using a commercial product containing an organic mercury compound. The commercial product, as is usual, would contain not the pure mercurial salt, but mercurial plus an unknown amount of inert "filler". The fact that he used a very large amount of the commercial substance, however, would mean a high concentration of the active constituent, and therefore his results could be compared with the results given in these studies.

Mercuric chloride has been used by several workers, including Mauer (1928), and Silbert (1925), and it was discovered by them that it had a toxic action on seeds. In this case it was probably the mercury ion that was the toxic agent.

The indifference of the beans to Mercurated o-chlorophenol, Mercurated o-nitrophenol and Tolyl mercuri acetate is difficult to explain, but results of a similar nature were obtained by Kiesselbach (1927), Sampson and Davies (1928) and Chippindale (1933). Thus the author's results are not without parallel. It seems that the toxic action of mercurial compounds, while probably due in the main

to the mercury cation, also depends on the substances with which the mercury ion is combined in the salt. How closely it is combined or held, and prevented from ionising, is probably the main factor. If it is tightly held then the compound is less likely to be poisonous than if the mercury is more free.

CONCLUSIONS.

(1) Poisoning of the radicle of *Vicia Faba* follows upon the soaking of the seed in Tolyl mercuri acetate. The poisoning results from contact with the salt which has reached the radicle by diffusion through that part of the testa immediately above the young rootlet.

(2) The occurrence of a varying number of cells, specially permeable to water, in the area of the testa above the embryo is the cause of the increase in wrinkling in that region, and this leads to the greater or less poisoning of the radicle.

(3) Total poisoning of the radicle is brought about as soon as the salt has passed through the micropyle, and has reached those parts of the embryo root not accessible to salt which has diffused through the testa.

(4) A bean seed, the coat of which has become cracked, is killed by Tolyl mercuri acetate, thus showing that an intact testa provides a certain amount of protection.

(5) The toxic effect of the salt is minimised by coating certain areas of the testa, notably the micropyle, with paraffin wax.

(6) A measure of protection is afforded to the beans if an inorganic salt providing a bivalent cation is added to the mercurial solution. Increased toxicity follows the addition of a salt containing a monovalent ion.

(7) The addition of an aqueous extract of excised seed-coat precipitates Tolyl mercuri acetate, and so reduced its toxicity towards beans which are subsequently soaked in it.

(8) The main constituent of a seed-coat extract is pyrogallol tannin.

(9) The removal of some of the salt from solution by the pyrogallol tannin of the coat must also take place in the testa as the salt passes through it, but sufficient salt to cause poisoning of the radicle always remains.

(10) The harmful effect of the salt is confined mainly to the radicle, the plumule being protected by its position between the cotyledons, and by its greater distance from the radicle.

(11) Growth of the plumule, subsequent to germination, is slowed down, probably as a reflex of reduction in root growth.

(12) Production of flowers on plants developed from partially poisoned embryos is delayed.

(13) The bean appears to be indifferent to the presence of Mercurated o-chlorophenol or Mercurated o-nitrophenol or Shirlan in the external fluid.

(14) There is a mild poisoning effect in beans soaked in a solution of Sodium-2-(hydroxymercuri) benzoate, reminiscent of that produced by Tolyl mercuri acetate.

(15) The toxic properties of these inorganic mercury salts are not dependent upon the presence of the Mercury ion alone: the substances with which it is combined, and the way they are combined, play no small part.

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TABLE I.

Effect of Saturated Solution of Tolyl mercuri acetate on Swelling Rate of Beans.

Bean No.	Wet Wgt.	Weight at undernoted periods of hours. 2	4	6	8	10	12	14	16	18	20	22	24	40	46	47½	64
1	1·21520	1·2180	1·21915	1·22124	1·22335	1·22560	1·23247	1·2430	1·26018	1·29506	1·3320	1·37715	1·43215	1·83580	1·95353	1·95530	2·3140
2	1·11515	1·11810	1·12303	1·12403	1·15008	1·16519	1·1850	1·20722	1·23414	1·27035	1·30541	1·33014	1·35121	1·51838	1·56472	1·57538	1·70532
3	1·35535	*															
4	1·02910	1·03020	1·03225	1·0350	1·03820	1·04324	1·04909	1·05708	1·06425	1·07504	1·08810	1·10316	1·11738	1·46494	1·86558		
5	1·3592	*															
6	1·01330	1·01710	1·02502	1·0390	1·06212	1·9210	1·12755	1·17215	1·22310	1·2870	1·36204	1·4310	1·4940	1·9950	2·17803	2·21213	2·37858
7	1·70925	*															
8	·91030	·9120	·91501	·91902	*												
9	1·20520	1·21030	1·22125	1·23506	1·24901	1·26470	1·28406	1·30015	1·30345	1·30720	1·41201	1·45002	1·48827	2·12510	2·48505	2·51815	2·82822
10	1·28030	1·2830	1·28313	1·28430	1·28604	1·28658	1·2890	1·29115	1·29134	1·29603	1·30	1·30330	1·31018	1·84706	2·08037	2·11614	2·54305
11	1·22905	1·22630	1·24001	1·27020	1·31105	1·35603	1·40001	1·44018	1·48870	1·52201	1·56003	1·60002	1·63517	1·93743	2·07515	2·1270	2·47722
12	·93425	·9371	*														
13	1·25605	1·26028	1·27003	1·2860	1·30605	1·32643	1·35041	1·37903	1·4140	1·44840	1·48411	1·52041	1·5580	1·9470	2·06605	2·0840	2·4310
14	1·61115	1·6160	1·62204	1·63232	1·65116	1·6720	1·73020	1·78412	1·83613	1·88035	1·91312	1·95045	1·98210	2·24137	2·32012	2·33532	2·49323
15	1·01415	1·03350	1·08030	1·11521	1·14318	1·17024	1·19117	1·21268	1·23205	1·25712	1·2850	1·30812	1·3310	1·54209	1·60739	1·61524	1·74028
AVERAGE	1·19617	1·20128	1·21163	1·22424	1·24202	1·26021	1·28390	1·30862	1·33473	1·36386	1·404302	1·43748	1·46995	1·84542	2·01760	2·05985	2·33010

*Seed-coat fractured during soaking.

TABLE II.

Effect of Distilled Water on Swelling Rate of Beans.

Bean No.	Wet Wgt.	Weight at undernoted periods of hours. 2	4	6	8	10	12	14	16	18	20	22	24
1	1·24535	*											
2	1·24330	1·3060	1·9930	2·2550	2·40501	2·4750	2·5250	2·5620	2·54602	2·62615	2·6530		2·6950
3	1·1520	1·1560	1·2210	1·34710	1·470	1·6410	1·83702	2·04030	2·43804	2·60535	2·73701	2·83524	2·90325
4	·98535	·99130	1·04009	1·11801	1·119709	1·3810	1·6870	1·770	1·830	1·85003	1·90015	1·95108	1·9870
5	1·05215	*											
6	1·46790	1·44102	1·5360	1·610	1·6960	1·9470	2·3510	2·54205	2·81301	2·9550	3·0362	3·15060	3·1720
7	1·2090	*											
8	1·47005	1·48210	1·59215	1·7830	1·96001	2·18270	2·71502	2·8510	2·9520	3·0140	3·060	3·10303	3·10350
9	1·17500	1·17910	*										
10	1·0740	1·07732	*										
11	1·14410	1·14801	1·19416	1·28601	1·42203	1·54808	1·68824	1·78526	1·88005	2·01501	2·120	2·22205	2·29605
12	1·230	1·2470	1·34206	1·67706	2·290	2·4910	2·5240	2·5168	2·5670	2·6850	2·810	2·83010	2·8520
13	1·1580	1·16112	1·16908	1·1930	1·24507	1·33060	1·43206	1·9650	2·14010	2·24002	2·3080	2·60725	2·68704
14	1·14225	*											
15	1·63130	1·63168	1·650	1·69707	1·7560	1·83451	1·95103	2·1040	2·37550	3·13540	3·36503	3·5246	3·550
AVERAGE	1·27693	1·29097	1·41526	1·55181	1·71569	1·87608	2·07893	2·23788	2·39908	2·56955	2·66548	2·77792	2·80509

*Seed-coat fractured during soaking.

TABLE III.

Effect of Saturated Solution of Mercurated o-chlorophenol on Swelling Rate of Beans.

Bean No.	Wet Wgt.	Weight at undernoted periods of hours. 2	4	6	8	10	12	26	28	30
1	1·4550	1·4580	1·46045	1·46614	1·47413	1·53608	1·68115	3·052	3·06338	3·07508
2	1·09230	1·09410	1·1002	1·15225	1·2230	1·31010	1·41325	2·33245	2·34715	2·35835
3	1·45225	1·45435	1·4830	1·57023	1·65517	1·76022	1·86510	3·01608	3·06715	3·1100
4	1·34335									
5	1·30330									
6	1·13620	1·13633	1·13829	1·14003	1·14105	1·14218	1·14225	1·18240	1·200616	1·23524
7	1·23740									
8	1·17221									
9	1·21123	1·21402	1·22329	1·26908	1·32810	1·39315	1·460	2·50029	2·52504	2·5470
10	1·2880	1·18960	1·19305	1·19609	1·20020	1·20014	1·3220	2·650	2·61517	2·67413
11	1·08818	1·10080	1·13328	1·17830	1·22510	1·28305	1·34206	2·66002	2·650	2·6760
12	1·08145									
13	1·01115	1·01505	1·02525	1·1170	1·2510	1·34008	1·44835	2·63030	2·25319	2·32713
14	1·00802	1·08910	1·01020	1·01310	1·01716	1·02214	1·02812	2·53815	2·56764	2·590
15	1·07704	1·08507	1·1420	1·2320	1·30810	1·4010	1·4610	1·77005	1·83635	1·90742
AVERAGE	1·17194	1·17564	1·19088	1·23342	1·28230	1·33971	1·40693	2·36317	2·41312	2·45004

TABLE IV.

Effect of Distilled Water on Swelling Rate of Beans.

Bean No.	Wet Wgt.	Weight at undernoted periods of hours. 2	4	6	8	10	12	26	28	30
16	1·03740	1·05217	1·10902	1·2120	1·33015	2·18304	2·31014	2·43535		
17	1·06140	1·06213	1·06230	1·06325	1·06326	1·06342	1·06625	1·33725	1·40629	1·48744
18	1·10030	1·10040	1·1370	1·21705	1·29404	1·3750	1·450	2·48839	2·5150	2·5330
19	1·23360									
20	1·27415	1·28020	1·33810	1·55505	1·83210	2·00105	2·11070	2·8060	2·8410	2·87614
21	1·27002	1·27215								
22	1·06914	1·0740	1·10308	1·23205	1·38845	1·53215	1·92214	2·61207	2·63145	2·64804
23	1·10936	1·1160	1·14505	1·22350	1·37810	1·52610	1·74507	2·4550	2·48001	2·50
24	1·2870	1·28935	1·30510	1·35015	1·4160	1·50835	1·63401	2·96020	2·98206	3·00311
25	1·38515	1·39008	1·40525	1·55170	1·69011	1·86810	2·22004	2·93806	2·96104	2·98328
26	1·23635	1·2390	1·24115	1·24520	1·2720	2·1230	2·58816	2·86209	2·87506	2·890
27	1·0010	1·0040	1·01320	1·06610	1·1620	1·2760	1·4370	2·42733	2·44470	2·46211
28	1·11810	1·28210	1·44604	1·82760	1·9840	2·09204	2·18608	2·550		
29	1·14458	1·14625	1·15020							
30	1·1384									
AVERAGE	1·15903	1·17177	1·20903	1·32260	1·43729	1·6820	1·87405	2·53379	2·57099	2·59812

TABLE V.

Effect of Saturated Solution of Mercurated o-nitrophenol on Swelling Rate of Beans.

Bean No.	Wet Wgt.	Weight at undernoted periods of hours. 2	4	6	8	10	12	26	28	30	32
1	1·26020	1·26511	1·28505	1·35006	1·46218	1·58304	1·710	3·04802	3·08501	3·11214	3·13206
2	1·03915	1·04415	1·07510	1·13011	1·21002	1·31008	1·4230	2·20706	2·24204	2·28504	2·310
3	1·0280	1·02912	1·03105	1·03545	1·0470	1·0630	1·08417	1·67303	1·80014	1·4890	2·0710
4	1·02435	1·02914	1·04308	1·0750	1·13512	1·2350	1·8320	2·13805	2·1540	2·16702	2·17626
5	1·0640										
6	1·18835	1·19335	1·25996	1·37712	1·4980	1·63712	1·9970				
7	1·01613										
8	1·03170	1·0350	1·6520	2·05010	2·53010	2·27505	2·30726	2·5110	2·52318	2·53506	2·5430
9	1·11514	1·11615	1·11804	1·12123	1·12360	1·16412	2·20535	2·2210	2·31001	2·38616	2·41712
10	1·01812	1·01908	1·02007	1·02144	1·0280	1·04515	1·18808	2·29203	2·2980	2·30603	2·3110
11	1·16203	1·17327	1·22507	1·3340	1·46706	1·79303	1·9330	2·39324	2·46116	2·51716	2·55035
12	1·22735	1·25311	1·37511	1·95714	2·27708	2·43105	2·55018	2·71307	2·73209	2·750	2·76080
13	1·01535	1·0180	1·02012	1·02317	1·02801	1·03613	1·04801	2·16506	2·42735	2·50513	2·54005
14	1·47240	1·47904	1·5180	1·58312	1·66504	1·76310	1·88314	3·21608	3·31806	3·42728	3·49206
15	1·06134										
AVERAGE	1·13185	1·13788	1·21855	1·33816	1·45593	1·52799	1·76343	2·41615	2·48646	2·53364	2·57306

TABLE VI.

Effect of Distilled Water on Swelling Rate of Beans.

Bean No.	Wet Wgt.	Weight at undernoted periods of hours. 2	4	6	8	10	12	26	28	30	32
16	1·02530	1·02612	1·02710	1·02711	1·02732	1·0280	1·02832	1·03512	1·03620	1·03828	1·04427
17	1·00825	1·0150	1·05724	1·17724	1·29524	1·43306	1·55524	2·34011	2·55306	2·36706	2·37221
18	1·01040	1·02334									
19	1·3150	1·3170	1·31807	1·31406	1·32401	1·32306	1·32734	2·57404	2·6140	2·77701	2·84104
20	1·11436	1·11810	1·13221	1·19107	1·2890	1·4520	1·56608	2·71004	2·72720	2·7416	2·75412
21	1·02915	1·04412	1·17005	1·31904	1·87050	2·11124	2·18038	2·25736	2·2610	2·2660	2·27201
22	1·33705	1·34514	1·40523	1·52602	1·64304	2·07202	2·3530	3·00511	3·02704	3·0650	3·06712
23	1·04555	1·0490	1·05724	1·09905	1·1730	1·49307	1·91006	2·34522	2·40605	2·41504	2·42306
24	1·43735	1·4440	1·5020	1·59103	1·72502	1·81521	1·90011	2·9470	3·1070	3·28213	3·4020
25	1·1050	1·10836									
26	1·16206	1·17532	1·21321	1·32310	1·43530	1·63816	1·86105	2·42217	2·57303	2·65502	2·69250
27	1·18435	1·19111	1·231414	1·31208	1·40523	1·51824	1·89318				
28	1·23723	1·25420	1·3224	1·40714	1·4980	1·62001	1·86308	2·73006	2·74312	2·76303	2·7750
29	1·16363	1·1680	1·1730	1·18524	1·22813	1·31424	1·45313	2·73538	2·74635		
30	1·31924										
AVERAGE	1·17161	1·17893	1·21764	1·28935	1·40932	1·56513	1·7409	2·46833	2·50856	2·53596	2·56453

TABLE VII.

Effect of Saturated Solution of Tolyl mercuri acetate on Germination.

Date of Sowing: 10th Oct. 1933.

Temperature of Solution: 17°C.

No. of Hours of Soaking.	No. of Beans sown.	Percentage Germination on the undernoted dates.							
		13TH Oct.	14TH Oct.	15TH Oct.	16TH Oct.	17TH Oct.	18TH Oct	19TH Oct.	20TH Oct.
½	20	70	90	95					
1	18	50	94	94					
2	20	65	100						
4	20	50	95	95					
6	17	24	71	100					
8	19	26	79						
24	20		10	30	35			40	45
26	19		11	16			21	26	32
30	18			6				12	
48	20								

TABLE VIII.

Effect of Distilled Water on Germination.

Date of Sowing: 10th Oct. 1933.

Temperature of Water: 17°C.

No. of Hours of Soaking.	No. of Beans sown.	Percentage Germination on the undernoted dates.							
		13TH Oct.	14TH Oct.	15TH Oct.	16TH Oct.	17TH Oct.	18TH Oct.	19TH Oct.	20TH Oct.
½	20	35	95						
1	20	45	95	100					
2	19	68	90	100					
4	20	60	90		95				
6	20	80	90	100					
8	20	90	95						
24	19	64	95	100					
26	20	70	85	100					
30	20	60	85	100					
48	20	45	85	100					

TABLE IX.

Untreated Beans.

Date of Sowing: 10th Oct. 1933.

Ref.No.	No.of Beans sown.	Percentage Germination on the undernoted dates.							
		13TH Oct.	14TH Oct.	15TH Oct.	16TH Oct.	17TH Oct.	18TH Oct.	19TH Oct.	20TH Oct.
1	20	30	90						
2	20	65	100						
3	18	50	100						
4	20	45	95	100					
5	20	45	100						
6	20	30	95		100				
7	19	0	21	94					
8	20	0	40	100					
9	20	0	40	80	95				
10	20	0	0	80	100				

TABLE X.

Effect of Saturated Solution of Tolyl mercuri acetate on Germination.

Date of Sowing: 17th Oct. 1933.

Temperature of Solution: 16.5°C.

No. of Hours of Soaking.	No. of Beans sown.	Percentage Germination on the undernoted dates.							
		20TH Oct.	21ST Oct.	22nd Oct	23RD Oct.	24TH Oct			
¼	20	0	75	90	100				
½	20	10	90	95	100				
¾	20	10	75	90	100				

TABLE XI.

Effect of Distilled Water on Germination.

Date of Sowing: 17th Oct. 1933.

Temperature of Water: 17°C.

No. of Hours of Soaking.	No. of Beans sown.	Percentage Germination on the undernoted dates.							
		20TH Oct.	21ST Oct.	22ND Oct.	23RD Oct.	24TH Oct.			
¼	20	0	80	95	100				
½	20	15	90	95	100				
¾	20	25	75	85	95				

TABLE XII.

Untreated Beans.

Date of Sowing: 17th Oct. 1933.

Ref. No.	No. of Beans sown.	Percentage Germination on the undernoted dates.							
		20TH Oct.	21ST Oct.	22ND Oct.	23RD Oct.	24TH Oct.			
11	20	0	80	95	100				

TABLE XIII.

Effect of Saturated Solution of Tolyl mercuri acetate on Germination.

Date of Sowing: 27th Nov. 1933.

Temperature of Solution: 16.5°C.

No. of Hours of Soaking.	No. of Beans sown.	Percentage Germination on the undernoted dates.							
		4TH Dec.	5TH Dec.	6TH Dec.	7TH Dec.	8TH Dec.	9TH Dec.		
½	20	0	60	90		95			
2	20	10		50	70	75			
4	20	5	15	20	50				
6	20	0	0	10					
8	20	0	10	15	25				
24	20	0	0	0					
30	20	0	0	5	5				
48	20	0	0	0					

TABLE XIV.

Effect of Distilled Water on Germination.

Date of Sowing: 27th Nov. 1933.

Temperature of Water: 17°C.

No. of Hours of Soaking.	No. of Beans sown.	Percentage Germination on the undernoted dates.							
		4TH Dec.	5TH Dec.	6TH Dec.	7TH Dec.	8TH Dec.	9TH Dec.		
½	20	5	60	90		100			
2	20	10	90						
4	20	25	85			95			
6	20	15	65		75	85			
8	20	5	95	100					
24	20	0	80	80					
30	20	5	80	90					
48	20	0	70	80	95				

TABLE XV.

Untreated Beans.

Date of Sowing.	No. of Beans sown.	Percentage Germination on the undernoted dates.							
		5TH DEC.	6TH DEC.	7TH DEC.	8TH DEC	9TH DEC			
27TH NOV.	20	70	100						
28TH NOV.	20	0	65	95					
29TH NOV	20	0		60	90				

TABLE XVI.

Effect of Diluted Solution of Tolyl mercuri acetate on Germination.

Date of Sowing: 25th April 1934.

Temperature of Solution: 18°C.

No. of Hours of Soaking.	No. of Beans sown.	Percentage Germination on the undernoted dates.							
		2ND MAY	3RD MAY	4TH MAY	5TH MAY	6TH MAY	7TH MAY	8TH MAY	9TH MAY
2	20	5	50	80	90	100			
4	20		40	75	80	90			95
6	20		30	70	85	90			100
8	20		15	70	80			85	85
24	20				10	20	25	30	35
30	20					5	10	15	25
48	20							10	10

TABLE XVII.

Effect of Distilled Water on Germination.

Date of Sowing: 25th April 1934.

Temperature of Water: 17.5°C.

No. of Hours of Soaking.	No. of Beans sown.	Percentage Germination on the undernoted dates.							
		2ND MAY	3RD MAY	4TH MAY	5TH MAY	6TH MAY	7TH MAY	8TH MAY	9TH MAY
2	20		45	100					
4	20	15	55	85	90	95			
6	20	20	65	85		90		100	
8	20	10	55	90	95				
24	20		40	85		90			
30	20		10	45	60	85	90		100
48	20			10	65	70	80	95	

TABLE XVIII.

Time of Soaking.	Type of Seedling.
2 hours in salt.	100% normal seedlings.
2 hours in water.	100% normal seedlings.
4 hours in salt.	55% normal seedlings, 45% with normal plumule and curved, stumpy root.
4 hours in water.	95% normal seedlings, 5% with normal root and broken plumular bud, the latter caught between the cotyledons and broken off.
6 hours in salt.	60% normal seedlings, 40% with normal plumule and curved, affected root.
6 hours in water.	95% normal seedlings, 5% with thin starved root and shoot.
8 hours in salt.	45% fairly normal seedlings, 40% with affected root, 15% non-germination.
8 hours in water.	90% normal seedlings, 5% of thin and starved appearance, 5% with normal root and plumular bud broken off.
24 hours in salt.	40% with normal plumule and short, curved stumpy root, 60% non-germination.
24 hours in water.	90% normal seedlings, 10% with thin root and normal developing plumule.
30 hours in salt.	30% with normal, though short, plumule, and with badly affected, curved, thin root, 70% non-germination.
30 hours in water.	90% normal seedlings, 10% with thin root and normal plumule.
48 hours in salt.	20% with normal plumule and short, stumpy root, 80% non-germination.
48 hours in water.	80% normal seedlings, 20% with slightly twisted root, and normal plumule.

TABLE XIX.

Relationship between Increase in Weight of Beans soaked in Saturated Solution of Tolyl mercuri acetate and Power of Germination.

Soaking commenced 29th March 1934.

Bean No.	Dry Wgt.	Weight after 18 hrs	Increase in Weight	Percentage increase in Weight	Date of Germination	Type of Seedling
1.	1·25534	1·56918	·31384	25		Short, stumpy root and developing plumule
2	1·35122	1·9650	·61378	45·2		No germination.
3	1·79423	1·92804	·13281	7·4	7TH APRIL	Normal
4	1·2223	1·2720	·0497	4·5		Normal germination commencing.
5.	1·00704	1·0110	·00346	·39	7TH APRIL	Normal germination, short root.
6	1·25724	1·56421	·30697	24·4		Curved root, plumule developing.
7	1·13530	2·05605	·92075	81·3		Root brown, discoloured, and not growing.
8	1·24804	*2·75607	1·50803	120·8		No germination
9	1·06140	1·2070	·14560	13·7		Short, curved root, plumule developing.
10	1·32827	*2·44105	1·11278	83·7		No germination.
11	1·34950	1·40902	·05952	4·39	7TH APRIL	Normal
12	1·19639	1·2950	·09861	8·24	8TH APRIL	Normal
13	1·52343	*3·260	1·73657	114		No germination.
14	1·53410	1·63302	·09892	6·43	8TH APRIL	Root curved, plumule normal.
15	1·22610	2·2880	1·06190	87·2		Coat burst, yellow root
16	1·16538	1·17415	·00877	·75		Normal
17	1·18244	1·18701	·00457	·38		Root normal, plumule almost through.
18	1·13535	2·52600	1·39065	122·5		No germination
19	1·45315	1·64525	·19010	13·1	7TH APRIL	Root twisted, plumule long and normal.
20	1·37635	*3·0270	1·65065	119·9		No germination.

*Seed-coat fractured during soaking.

TABLE XX.

Relationship between Increase in Weight of Beans soaked in Distilled Water and Power of Germination.

Soaking commenced 2nd April 1934.

Bean No.	Dry Wgt.	Weight after 18 hrs	Increase in Weight	Percentage increase in Weight	Date of Germination	Type of Seedling
1.	1·37422	1·84004	·46582	34·1	10TH APRIL	Normal
2	1·21328	1·53206	·31878	25·25	10TH APRIL	Normal
3	1·26240	* 2·59505	1·33265	105·7	11TH APRIL	Normal
4	1·15017	2·37707	1·22690	106·7	10TH APRIL	Normal
5	1·26712	1·70817	·44105	34·8	10TH APRIL	Normal
6	1·3515	1·43014	·07864	5·82	11TH APRIL	Normal
7	1·13436	1·93125	·79689	70·25	11TH APRIL	Normal
8	1·14505	* 2·31214	1·16709	101·9	10TH APRIL	Normal
9	1·08505	2·4460	1·36095	125·3	12TH APRIL	Normal
10	1·19020	2·32707	1·13687	95·5	10TH APRIL	Normal
11	1·57229	2·2020	·62971	40·	11TH APRIL	Normal
12	1·5340	1·60490	·07090	4·62	11TH APRIL	Normal
13	1·20012	1·20420	·00408	·34	12TH APRIL	Normal
14	1·58320	2·11704	·53384	33·6	11TH APRIL	Normal
15	1·41037	1·58611	·17574	12·46	11TH APRIL	Normal
16	1·10708	* 2·5330	1·42592	128·7	15TH APRIL	Normal
17	1·26102	2·4510	1·18998	94·3	9TH APRIL	Normal
18	1·11334	2·5270	1·41366	126·8	11TH APRIL	Normal
19	1·31531	1·34609	·03078	2·34		Normal
20	1·37737	2·7810	1·40363	101·9	10TH APRIL	Normal

*Seed-coat fractured during soaking.

TABLE XXI.

Relationship between Increase in Weight of Beans soaked in Saturated Solution of Tolyl mercuri acetate and Power of Germination.

Soaking commenced 24th April 1934.

Bean No.	Dry Wgt.	Weight after 6 hrs	Increase in Weight	Percentage increase in Weight	Date of Germination	Type of Seedling
1	1·53905	1·5430	·00395	·26		Normal
2	1·2490	1·25125	·0025	·18	3RD MAY	Normal
3	1·04540	1·0610	·01560	1·49	2ND MAY	Normal
4	1·34410	1·38530	·04120	3·06	2ND MAY	Normal
5	1·37823	1·4150	·03677	2·67	2ND MAY	Root slightly curved. Plumule normal
6	1·3370	1·38408	·04708	3·52	2ND MAY	Normal
7	1·18422	1·45006*	·26074	21·95	3RD MAY	Root affected, curved and twisted. Plumule normal.
8	1·41035	1·42212	·01177	·83	2ND MAY	Normal
9	1·60823	1·61905	·01082	·67	2ND MAY	Normal
10	1·38928	1·44004	·05076	3·66	2ND MAY	Normal
11	1·31235	2·2550*	·94265	71·85		No germination
12	1·37017	1·37620	·00603	·44	3RD MAY	Normal
13	1·38726	1·45816	·07090	5·18	3RD MAY	Root curved. Plumule normal.
14	1·53418	1·54418	·01000	·65	2ND MAY	Normal
15	1·58535	1·59504	·00969	·61	2ND MAY	Normal
16	1·28506	1·2890	·00394	·31	3RD MAY	Normal
17	1·45229	1·52404	·07175	4·94	1ST MAY	Normal
18	1·21305	1·23125	·02820	2·32	2ND MAY	Normal
19	1·29004	1·29813	·00809	·027	2ND MAY	Normal
20	1·0310	1·0380	·0070	·68	2ND MAY	Normal

*Seed-coat fractured during soaking.

TABLE XXII.

Relationship between Increase in Weight of Beans soaked in Saturated Solution of Tolyl mercuri acetate and Power of Germination.

Soaking commenced 4th May 1934.

Bean No.	Dry Wgt.	Weight after 6 hrs	Increase in Weight	Percentage increase in Weight	Area of Wrinkling	Date of Germination
1	1·36635	1·37005	·00370	·271		12TH MAY
2	1·22901	1·28725	·05824	4·74	BEHIND THE STROPHIOLE	11TH MAY
3	1·290	1·2940	·0040	·31		12TH MAY
4	1·36210	1·36813	·00603	·44		12TH MAY
5	1·11901	1·13324	·01423	1·19		12TH MAY
6	1·24032	1·4080	·16768	13·51	HILAR + TOWARDS BACK OF THE BEAN	
7	1·34031	1·51820	·17789	13·27	HILAR + TOWARDS BACK OF THE BEAN	
8	1·15513	1·16109	·00596	·52		12TH MAY
9	1·54020	2·53905	·99885	86·4	TOTAL PERIPHERAL WRINKLING	
10	1·39843	1·6690	·27057	13·98	ABOVE EMBRYO + ALL OVER ONE SIDE	
11	1·64515	1·82502	·17987	10·94	ABOVE EMBRYO + ROUND PERIPHERY	13TH MAY
12	1·19045	1·21320	·02275	1·91	STROPHIOLE	12TH MAY
13	1·06535	1·06712	·0177	·17		17TH MAY
14	1·55240	1·55610	·00370	·24		13TH MAY
15	1·20535	1·25602	·00067	·059	STROPHIOLE	12TH MAY
16	1·49125	1·77706	·28581	19·16	EARLY STAGE OF TOTAL WRINKLING	14TH MAY
17	1·06295	1·17804	·11579	10·9	ABOVE EMBRYO + SIDE OF ONE COTYLEDON	12TH MAY
18	1·30310	1·32015	·01705	1·31	CENTRE OF ONE SIDE	12TH MAY
19	1·08215	1·08405	·00190	·18		12TH MAY
20	1·56501	2·8510*	1·38599	88·6		

*Seed-coat fractured during soaking.

TABLE XXIIa.

Relationship between Increase in Weight of Beans soaked in Saturated Solution of Tolyl mercuri acetate and Power of Germination.

(Continuation of TABLE XXII).

	Type of Seedling.
1.	Normal seedling.
2.	Normal seedling.
3.	Normal seedling.
4.	Normal seedling.
5.	Normal seedling.
6.	Seedling with short, curved root and normal plumule.
7.	Seedling with short, stumpy, but straight root, and normal plumule.
8.	Normal seedling.
9.	Seedling with brown, curved root, and no plumule visible.
10.	No germination.
11.	Seedling with short, curved root and normal plumule.
12.	Normal seedling.
13.	Normal seedling.
14.	Normal seedling.
15.	Normal seedling.
16.	Seedling with curved root and normal plumule.
17.	Seedling with curved root and normal plumule.
18.	Normal seedling.
19.	Normal seedling.
20.	No germination.

TABLE XXIII.

Relationship between Increase in Weight of Beans soaked in Distilled Water and Power of Germination.

Soaking commenced 1st May 1934.

Bean No.	Dry Wgt.	Weight after 6 hrs	Increase in Weight	Percentage increase in Weight	Date of Germination	Type of Seedling
1.	1·25235	1·2530	·0065	·052	11TH MAY	Normal
2	1·29401	1·30830	·01429	1·10	10TH MAY	Normal
3	1·0930	1·09805	·00505	·46	10TH MAY	Normal
4	1·31535	1·31830	·00295	·22	10TH MAY	Normal
5	1·55320	2·72010	1·16690	75·3	9TH MAY	Normal
6	1·0930	1·11901	·02601	2·38	10TH MAY	Normal
7	1·22740	1·37705	·14965	12·2	9TH MAY	Normal
8	1·24808	1·310	·06192	4·96	9TH MAY	Normal
9	1·22708	1·34605	·11897	9·66	9TH MAY	Normal
10	1·01537	1·01911	·00374	·36	10TH MAY	Normal
11	1·09615	1·10620	·01005	·92	12TH MAY	Normal
12	1·35630	1·5710	·21470	15·8	9TH MAY	Normal
13	1·19117	1·2270	·03583	3·05	9TH MAY	Normal
14	1·19145	*2·3230	·13155	11·04	9TH MAY	Normal
15	1·16609	1·2620	·09591	8·23	9TH MAY	Normal
16	1·09127	*2·19612	1·10485	101·2		Normal
17	1·49620	1·56904	·07284	4·87	9TH MAY	Normal
18	1·35016	1·49801	·14785	10·95	9TH MAY	Normal
19	1·73425	*2·91520	1·54095	112·1		Diseased Bean
20	1·46531	*2·39520	·92989	63·44	9TH MAY	Normal

*Seed-coat fractured during soaking.

TABLE XXIV.

Effect of Saturated Solution on Tolyl mercuri acetate on the Germination and subsequent Development of Beans, compared with that of Beans soaked in Distilled Water, and untreated Beans.

Date of Sowing: 29th March 1934.

Time of Soaking: 12 hours.

Type of Solution	No. of Beans sown.	Percentage Germination on the undernoted dates.												
		18th April	19th April	20th April	21st April	22nd April	23rd April	24th April	25th April	26th April	27th April	28th April	29th April	30th April
Tolyl mercuri acetate	20						10	25	40	45	55		75	85
Distilled Water	18	5·5	11·1	22·2	55·5	72·2		83·3			88·8		94·4	
Untreated	10		20		50		70	90	90			100		

TABLE XXV.

Effect of Saturated Solution of Tolyl mercuri acetate on Germination of Beans with Micropylar Area waxed, compared with unwaxed Beans.

Date of Sowing: 19th Oct. 1933.

Type of Bean	No. of Hrs. of Soaking	No. of Beans sown	Percentage Germination on the undernoted dates.						
			23RD Oct.	24TH Oct.	25TH Oct.	26TH Oct.	27TH Oct.	28TH Oct.	
Waxed	24	17	51	76	88·3				
	48	20		5	35	75			
Unwaxed	24	20	25	40	45				
	48	20	0	0					

TABLE XXVI.

Effect of Saturated Solution of Tolyl mercuri acetate on Germination of Beans with various Areas waxed, compared with unwaxed Beans.

Date of Sowing: 25th Oct. 1933.

Area of Bean Waxed	No. of Hrs. of Soaking	No. of Beans sown	Percentage Germination on the undernoted dates.						
			30TH Oct.	31ST Oct.	1ST Nov.	2ND Nov.	3RD Nov.		
Hilum	28	11	27	72·8					
Micropyle	28	14	79	85·3					
Wax float-ed off	28	8	25	37·5					
Unwaxed	28	16	12·5	18·7	31·6				

TABLE XXVII.

Effect of Saturated Solution of Tolyl mercuri acetate on Germination of Beans with Micropyle waxed.

Date of Sowing: 11th Dec. 1934.

Time of Soaking: 24 hours.

Total Percentage of Germination: 93.6.

Type of Seedling.

1. 6.2% normal seedlings.
2. 37.5% with thin, whip-like root, coming from a thick root base, and normal plumule.
3. 18.9% with short, curved root and normal plumule.
4. 31.2% with short, stout root, in some cases the thin growing point was developing; normal plumule just bursting the coat.
5. 6.2% non-germination.

TABLE XXVIII.

Effect of washing Beans - soaked in Saturated Solution of Tolyl mercuri acetate - on subsequent Germination.

Date of Sowing: 18th Dec. 1933.

Type of Beans sown	No. of Hours of soaking	No. of Beans sown	Percentage of Germination
Waxed	4	20	80
"	6	18	67
"	24	20	35
Unwaxed	24	36	97

TABLE XXIX.

Type of Seedling produced by the waxed and unwaxed Beans after soaking for 24 hours

Waxed Beans: 30.5% with root very slightly affected, but not fattened by salt, and normal plumule.

Unwaxed Beans: 0%.

Waxed Beans: 50% with curved root and showing signs of developing into whip-like root; plumule normal.

Unwaxed Beans: 22.2% with curved root and showing signs of developing into whip-like root; plumule normal.

Waxed Beans: 16.7% with short, curved root, and normal plumule.

Unwaxed Beans: 16.7% with curved root and normal plumule.

Waxed Beans: 2.8% non-germination.

Unwaxed Beans: 61.1% non-germination.

TABLE XXX.

Effect of Saturated Solution of Tolyl mercuri acetate on Germination.

Date of Sowing: 30th Jan. 1934.

No. of Hours of Soaking.	No. of Beans sown.	Percentage Germination on the undernoted dates.							
		7TH FEB.	8TH FEB.	9TH FEB.	10TH FEB.				
2	20	25	60	70					
8	20		5	10					
24	20								

TABLE XXXI.

Effect of Saturated Solution of Tolyl mercuri acetate plus .15% Solution of Sodium sulphate on Germination.

No. of Hours of Soaking.	No. of Beans sown.	Percentage Germination on the undernoted dates.							
		7TH FEB.	8TH FEB.	9TH FEB.	10TH FEB.				
2	20	30	60	70					
8	20		1						
24	20								

TABLE XXXII.

Effect of Saturated Solution of Tolyl mercuri acetate plus .15% Solution of Ferrous sulphate on Germination.

No. of Hours of Soaking.	No. of Beans sown.	Percentage Germination on the undernoted dates.							
		7TH FEB.	8TH FEB.	9TH FEB.	10TH FEB.				
2	20	5	40	45					
8	20								
24	20								

TABLE XXXIII.

Effect of Saturated Solution of Tolyl mercuri acetate plus .15% Solution of Calcium sulphate on Germination.

No. of Hours of Soaking.	No. of Beans sown.	Percentage Germination on the undernoted dates.							
		7TH FEB.	8TH FEB.	9TH FEB.	10TH FEB.				
2	20	15	35	65					
8	20			10					
24	20								

TABLE XXXIV.

The Several Types of Seedlings.

Treatment No. of Beans 20	Normal Root & Plumule	Thin Root & normal Plumule	Short curved Root & normal Plumule	Short straight Root & normal Plumule	Just germinated	Did not germinate
2 hrs. in Salt	35%	20%	45%			
Sodium sulphate	50%		40%	10%		
Ferrous sulphate	25%	10%	50%	15%		
Calcium sulphate	25%	10%	40%	25%		
8 hrs. in Salt		10%	90%			
Sodium sulphate			70%			30%
Ferrous sulphate			90%	5%		5%
Calcium sulphate		5%	75%	20%		
24 hrs. in Salt			5%	20%	20%	55%
Sodium sulphate				25%	10%	65%
Ferrous sulphate			5%	40%	15%	40%
Calcium sulphate			15%	15%	25%	45%

TABLE XXXV.

Effect of Satruated Solution of Tolyl mercuri acetate plus Coat Extract Solution on Germination.

Date of Sowing: 25th May 1934.

Type of Solution.	No. of Hrs. of Soaking.	No. of Beans sown.	Percentage Germination on undernoted dates.							
			1ST JUNE	2ND JUNE	3RD JUNE	4TH JUNE	5TH JUNE	6TH JUNE	7TH JUNE	8TH JUNE
Salt	7 HRS. 45 MINS.	15	6·6	26·6	46·6	53·3	73·3	80	86·6	
Salt plus Extract	7 HRS. 45 MINS.	15	20	73·3	80	86·6	93·3			
Salt plus Extract filtered	7 HRS. 45 MINS.	15	33·3	80		93·3				

TABLE XXXVI.

Time of Soaking: 7 hours 45 minutes.

Treatment.	Type of Seedling.
Salt Solution	60% with short, curved root and normal plumule, 33.3% with thin, whip-like root, coming from a thick base, and normal plumule, 6.7% non-germination.
Salt plus Extract	46.3% normal plants, 40.3% with thin, whip-like root, coming from a thick base, 6.7% with short curved root and normal plumule, 6.7% non-germination.
Salt plus Extract filtered	73.4% normal plants, 13.3% with slightly affect- root, 6.7% with short, curved, stumpy, stout root and normal plumule, 6.7% just commencing germination.

TABLE XXXVII.

Effect of a Saturated Solution of Mercurated o-chlorophenol on Germination

Date of Sowing: 30th Oct. 1933.

No. of Hours of Soaking.	No. of Beans sown.	Percentage Germination on the undernoted dates.							
		2ND Nov.	3RD Nov.	4TH Nov.	5TH Nov.	6TH Nov.	7TH Nov.	8TH Nov	
¼	20	20	65	100					
½	20	20	85	95	100				
1	17	6	65	94	100				
2	21	14	48	81	90	100			
4	19	6	47	84	84	100			
6	20	20	50	60	85	95			
8	20	15	80	95					
24	18	44	78	89					
30	20	45	75	85	95				
48	20	35	65	80	95	100			

TABLE XXXVIII.

Effect of Distilled Water on Germination.

Date of Sowing: 30th Oct. 1933.

No. of Hours of Soaking.	No. of Beans sown.	Percentage Germination on the undernoted dates.							
		1ST Nov.	2ND Nov.	3RD Nov.	4TH Nov	5TH Nov	6TH Nov	7TH Nov	8TH Nov
¼	20		15	30	55	95	100		
½	20		5	80	95	100			
1	20		15	60	95	100			
2	18		17	39	100				
4	20		25	60	85	90	100		
6	20		10	40	75	75	85		95
8	20		15	60	85	100			
24	20	5	55	60	75	95			
30	20		55	65	85			90	
48	20		35	65	80	90			

TABLE XXXIX.

Effect of Saturated Solution of Mercurated o-chlorophenol on Germination.

Date of Sowing: 18th Dec. 1933.

Temperature of Solution: 16.5°C.

No. of Hours of Soaking.	No. of Beans sown.	Percentage Germination on the undernoted dates.										
		25TH DEC.	26TH DEC.	27TH DEC.	28TH DEC.	29TH DEC.	30TH DEC	31ST DEC.	1ST JAN.	2ND JAN.	3RD JAN.	
2	20	70	80	85	90	45			100			
4	20	60	85			90				85		
6	20	45	85	90	95							
8	18	22	33	56	72	78			89			
24	20	30	55	85	90							
30	20	15	25	60	70	80	85					
48	20	10	15	25	45	90		100				

TABLE XL.

Effect of Distilled Water on Germination.

Date of Sowing: 18th Dec. 1933.

Temperature of Water: 16.5°C.

No. of Hours of Soaking.	No. of Beans sown.	Percentage Germination on the undernoted dates.										
		25TH DEC.	26TH DEC.	27TH DEC.	28TH DEC.	29TH DEC.	30TH DEC.	31ST DEC.	1ST JAN.	2ND JAN.	3RD JAN.	
2	20	60	70	75						80		
4	19	42	84	89	94							
6	20	55	75	80	90	95						
8	19	37	52	100								
24	20	60	80		95							
30	20	20	40	60	70				75			
48	20	10	40	60	75	95			100			

TABLE XLI.

Time of Soaking.	Type of Seedling.
2 hours in salt	100% normal seedlings.
2 hours in water	80% normal seedlings, 20% slightly abnormal.
4 hours in salt	95% normal seedlings, 5% smaller than the remainder.
4 hours in water	100% normal seedlings.
6 hours in salt	95% normal seedlings, 5% non-germination.
6 hours in water	95% normal seedlings, 5% non-germination.
8 hours in salt	80% normal seedlings, 20% with thin plumular stalk and short, though normal-looking, root.
8 hours in water	88.9% normal seedlings, 11.1% non-germination.
24 hours in salt	90% normal seedlings, 5% with abnormal plumule, 5% non-germination.
24 hours in water	95% normal seedlings, 5% non-germination.
30 hours in salt	85% normal seedlings, 15% with short root and normal plumule.
30 hours in water	85% normal seedlings, 10% with short root and normal plumule.
48 hours in salt	75% normal seedlings, 25% with normal root and slight, suppressed, lateral roots.
48 hours in water	100% normal seedlings.

TABLE XLII.

Effect of Saturated Solution of Mercurated o-nitrophenol on Germination.

Date of Sowing: 8th Jan. 1934.

Temperature of Solution: 15°C.

No. of Hours of Soaking.	No. of Beans sown.	Percentage Germination on the undernoted dates.							
		10TH JAN.	11TH JAN.	12TH JAN.	13TH JAN.	14TH JAN.	15TH JAN.	16TH JAN.	
2	20		35	60	95				
4	20		40	45	95	100			
6	20		50	80	100				
8	20	5	40	80	95				
24	20	10	70	90	95		100		
30	20		75	90	95		100		
48	20		25	50	70	90	95		

TABLE XLIII.

Effect of Distilled Water on Germination.

Date of Sowing: 8th Jan. 1934.

Temperature of Water: 15°C.

No. of Hours of Soaking.	No. of Beans sown.	Percentage Germination on the undernoted dates.							
		10TH JAN.	11TH JAN.	12TH JAN.	13TH JAN.	14TH JAN.	15TH JAN.	16TH JAN.	
2	20		55	95		100			
4	20	5	60	85	95				
6	20	10	40	85	90	100			
8	20	10	65	85	90	95			
24	20	25	60	85	95		100		
30	20		80	100					
48	20		45	85	95		100		

TABLE XLIV.

Effect of Saturated Solution of Shirlan on Germination.

Date of Sowing: 16th May 1934.

Temperature of Solution: 17°C.

No. of Hours of Soaking.	No. of Beans sown.	Percentage Germination on the undernoted dates.							
		23RD May	24TH May	25TH May	26TH May	27TH May	28TH May.		
2	20	20	90	95	100				
4	20	10	70	85	95				
6	20	5	60	90	100				
8	20		90	95		100			
24	20		90	95					
30	20	5	45	80	85	90			
48	20		20	86		85			

TABLE XLV.

Effect of Distilled Water on Germination.

Date of Sowing: 16th May 1934.

Temperature of Water: 17°C.

No. of Hours of Soaking.	No. of Beans sown.	Percentage Germination on the undernoted dates.							
		23RD May	24TH May	25TH May	26TH May	27TH May	28TH May		
2	20	15	80	85	90				
4	20	5	70	95					
6	20		55	95	100				
8	20		65	85	90				
24	20		70	90			95		
30	20	15	50	70	85				
48	20		40	75	80	85	90		

TABLE XLII.

Effect of Saturated Solution of Mercurated o-nitrophenol on Germination.

Date of Sowing: 8th Jan. 1934.

Temperature of Solution: 15°C.

No. of Hours of Soaking.	No. of Beans sown.	Percentage Germination on the undernoted dates.							
		10TH JAN.	11TH JAN.	12TH JAN.	13TH JAN.	14TH JAN.	15TH JAN.	16TH JAN.	
2	20		35	60	95				
4	20		40	95	95	100			
6	20		50	80	100				
8	20	5	40	80	95				
24	20	10	70	90	95		100		
30	20		75	90	95		100		
48	20		25	50	70	90	95		

TABLE XLIII.

Effect of Distilled Water on Germination.

Date of Sowing: 8th Jan. 1934.

Temperature of Water: 15°C.

No. of Hours of Soaking.	No. of Beans sown.	Percentage Germination on the undernoted dates.							
		10TH JAN.	11TH JAN.	12TH JAN.	13TH JAN.	14TH JAN.	15TH JAN.	16TH JAN.	
2	20		55	95		100			
4	20	5	60	85	95				
6	20	10	40	85	90	100			
8	20	10	65	85	90	95			
24	20	25	60	85	95		100		
30	20		80	100					
48	20		45	85	95		100		

TABLE XLVII.

Effect of .1% Solution of Sodium-2-(hydroxymercuri) benzoate on Germination.

Date of Sowing: 26th March 1934.

Temperature of Solution: 15°C.

No. of Hours of Soaking.	No. of Beans sown.	Percentage Germination on the undernoted dates.							
		2ND APRIL	3RD APRIL	4TH APRIL	5TH APRIL	6TH APRIL	7TH APRIL		
2	20	65	95	100					
4	20	70	85	95		100			
6	20	40	95						
8	20	40	90	95		100			
24	20	10	45	55	60				
30	20		30	40		55			
48	20		10	15	25				

TABLE XLVIII.

Effect of Distilled Water on Germination.

Date of Sowing: 26th March 1934.

Temperature of Water: 15°C.

No. of Hours of Soaking.	No. of Beans sown.	Percentage Germination on the undernoted dates.							
		2ND APRIL	3RD APRIL	4TH APRIL	5TH APRIL	6TH APRIL	7TH APRIL		
2	20	50	90						
4	20	15	90	95	100				
6	20	30	90						
8	20	40	80	95	100				
24	20	5	65	85	100				
30	20	15	60	80	85				
48	20		30	60	75	90			

TABLE XLIX.

Time of Soaking.	Type of Seedling.
2 hours in salt.	100% normal seedlings.
2 hours in water.	100% normal seedlings.
4 hours in salt.	100% normal seedlings.
4 hours in water.	95% normal seedlings, 5% broken seedlings, i.e. with plumular bud broken off.
6 hours in salt.	95% normal seedlings, 5% with plumular bud diseased and undeveloped.
6 hours in water.	90% normal seedlings, 5% diseased beans, 5% with plumular bud diseased.
8 hours in salt.	95% normal seedlings, 5% with plumular bud broken off.
8 hours in water.	90% normal seedlings, 5% with poorly developed root, 5% with poor root and plumule.
24 hours in salt.	60% normal seedlings, 10% fairly normal but not developed, 5% with undeveloped root and normal plumule, 5% with short, curved root and normal plumule, 20% non-germination.
24 hours in water.	100% normal seedlings.
30 hours in salt.	50% normal seedlings, 20% with short, curved root and slightly developed plumule, 10% with developing root and no plumule, 5% with no root and developing plumule, 15% non-germination.
30 hours in water.	100% normal seedlings.
48 hours in salt.	15% normal seedlings, 10% with normal plumule, and short root, 75% non-germination.
48 hours in water.	90% normal seedlings, 10% showing commencement of normal germination.

G R A P H

and

F I G U R E S

I - 17

GRAPH: To show the increase in weight of beans soaked in saturated solutions of Tolyl mercuri acetate, Mercurated o-chlorophenol and Mercurated o-nitrophenol, using distilled water as a control in each case.

.49 cm. - 2 hours: .49 cm. - .I gram.

Tolyl mercuri acetate ——————————

Distilled water control ----------

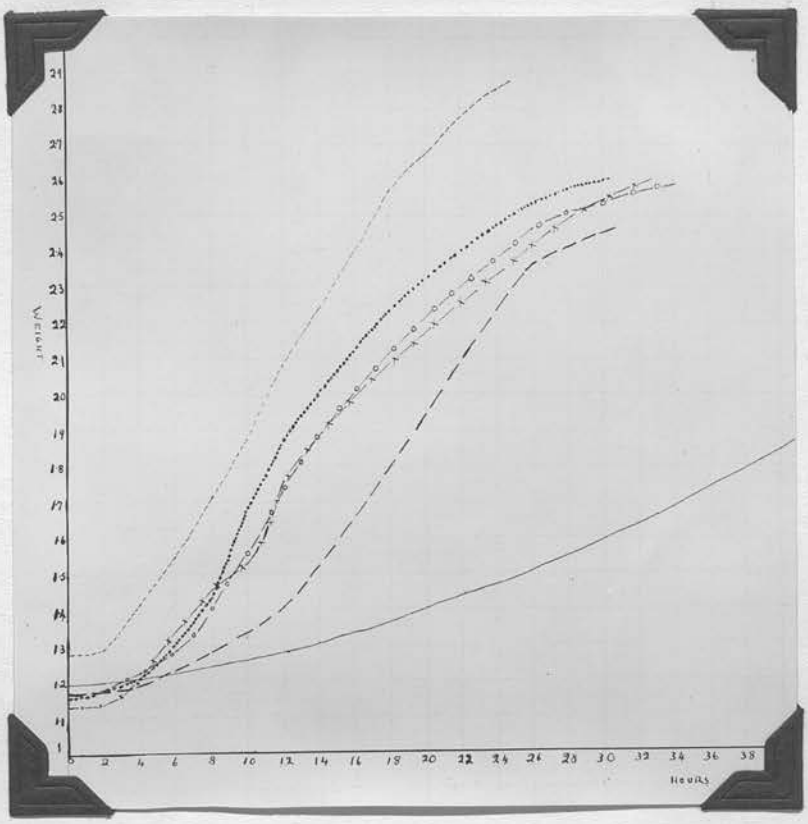
Mercurated o-chlorophenol — — — — — — — — —

Distilled water control .....

Mercurated o-nitrophenol - x - x - x - x - x

Distilled water control - o - o - o - o - o

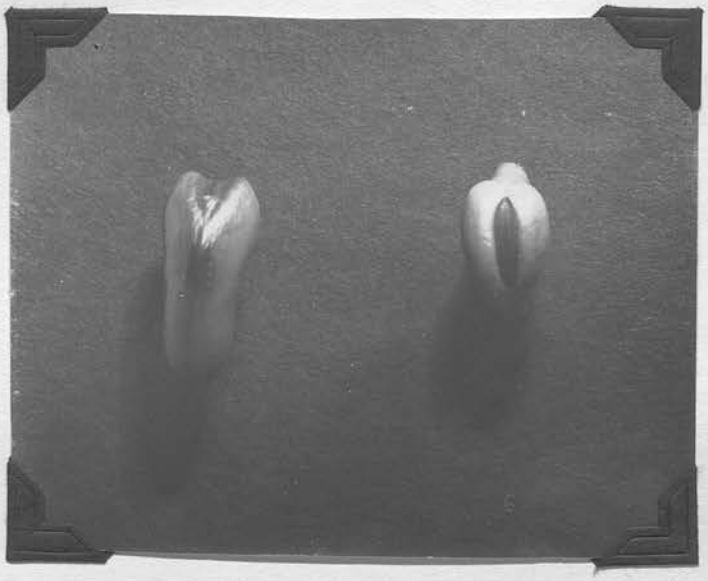
GRAPH.



FIGURES I & 2.

FIGURES 1 & 2.

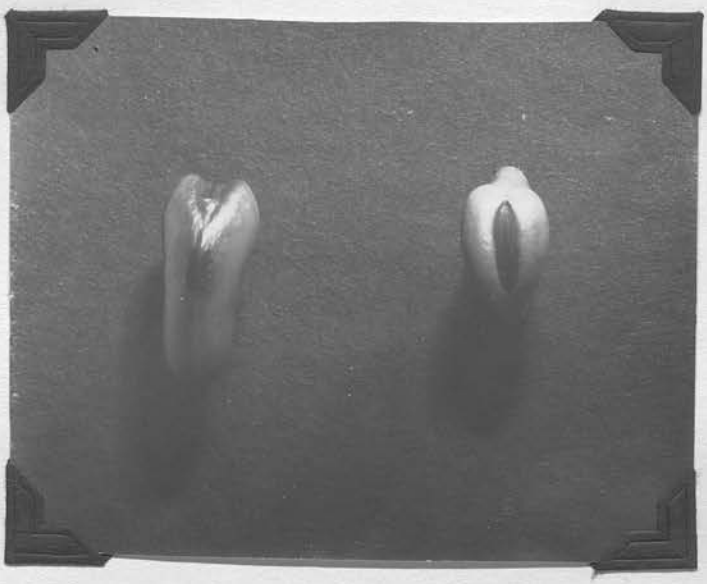
2.



1.

FIGURES 1 & 2.

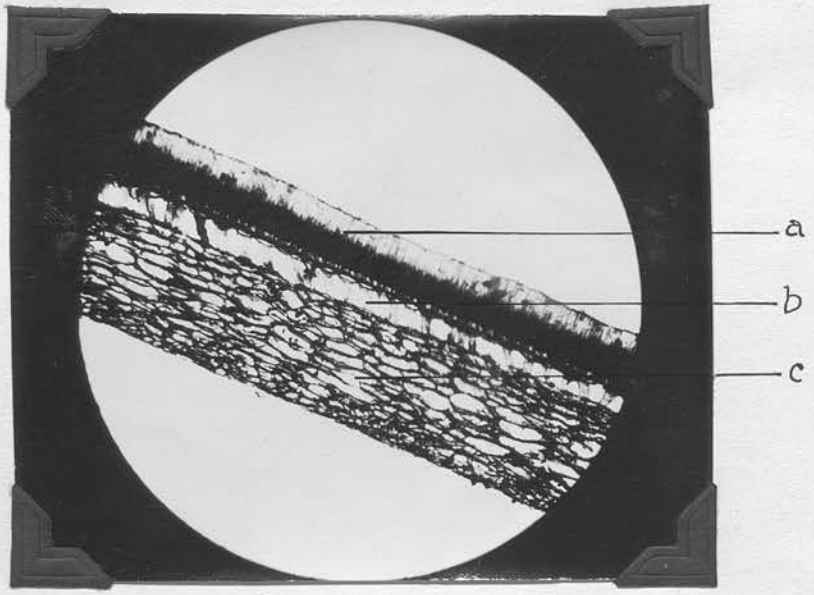
2.

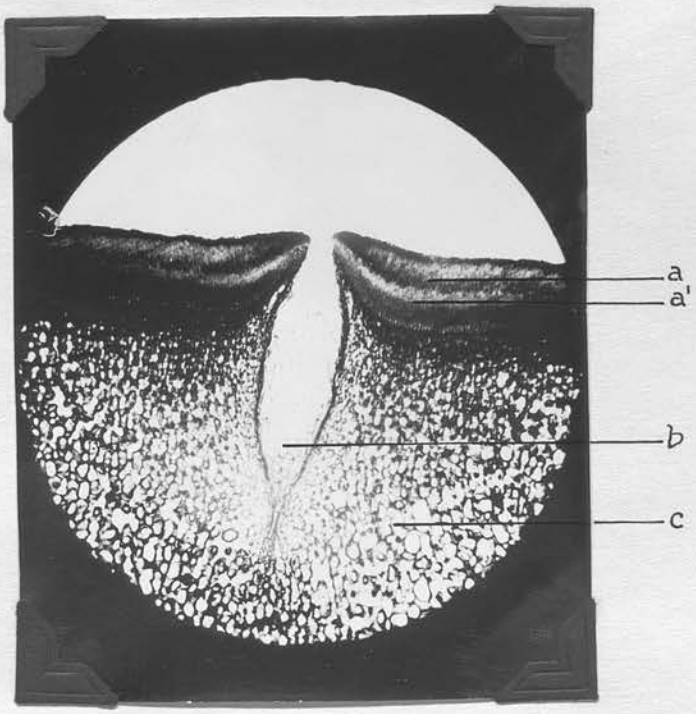


1.

FIGURES 3 & 4.

FIGURES 3 & 4.



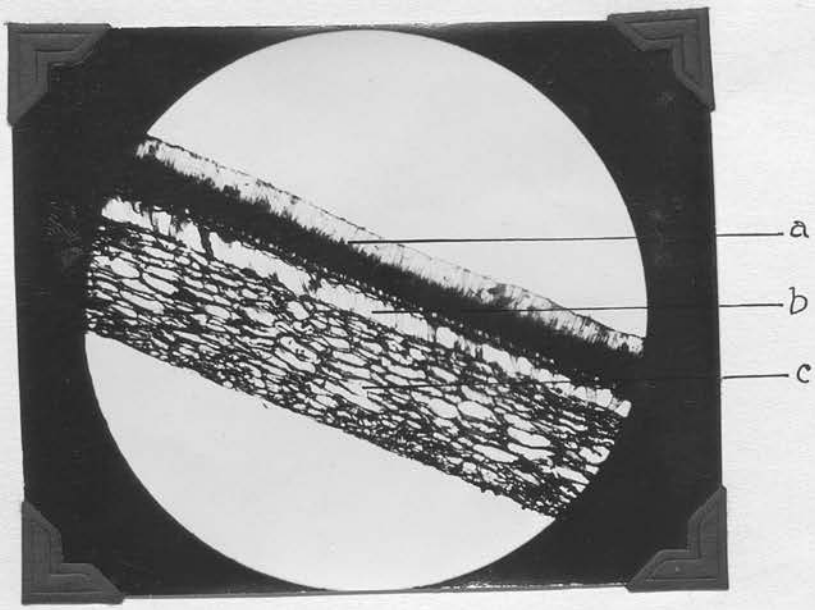


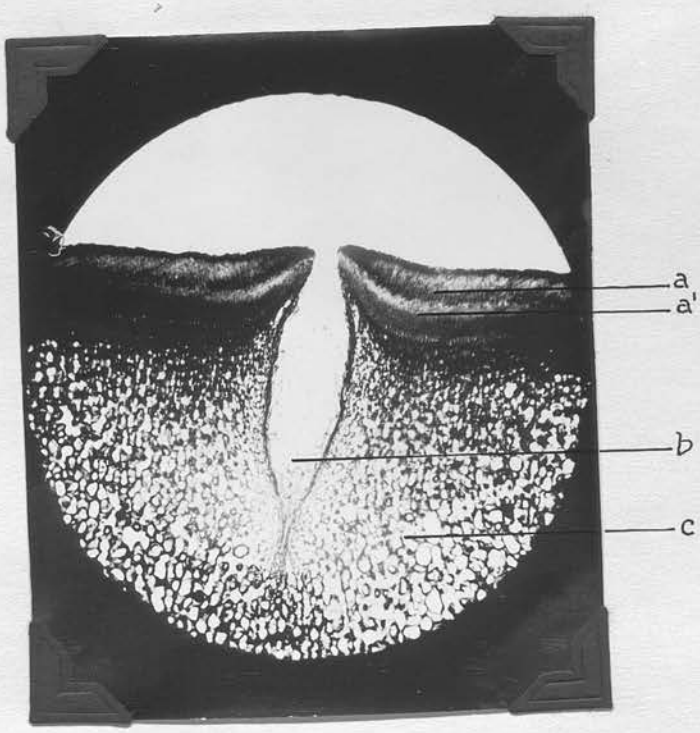
FIGURES 3 & 4.

Fig. 3. Transverse section of bean testa.
a. palisade layer; b. hour-glass cells;
c. nutrient layer. x 45.

Fig. 4. Transverse section through the hilum.
a. outer palisade layer; a'. inner palisade layer;
b. tracheid island; c. star-shaped parenchyma.
x 45.

FIGURES 3 & 4.





FIGURES 5 & 5a.

Fig. 5. Bean testa from the inside, showing the two dark-coloured, crescent-shaped areas to left and right of the radicle pocket.

Fig. 5a. Surface view of one of the crescent-shaped areas. x 45.

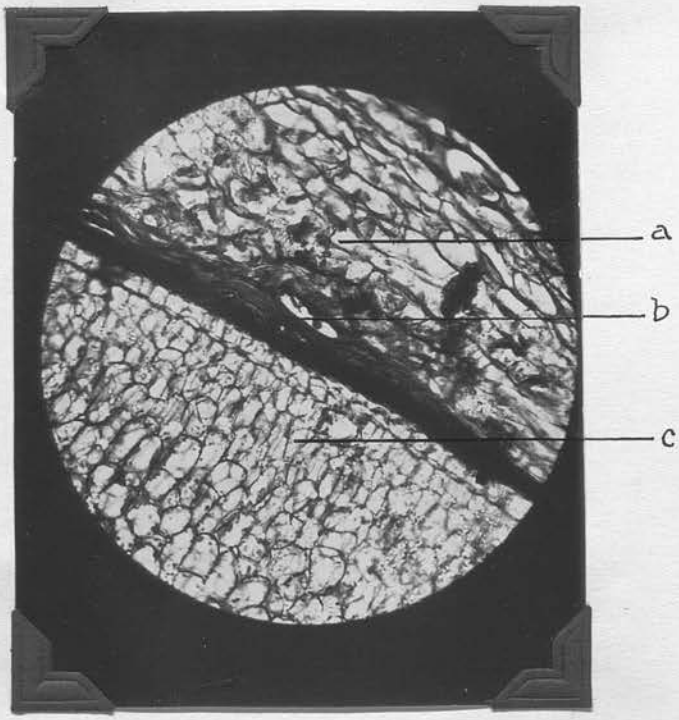
FIGURES 5 & 5a.

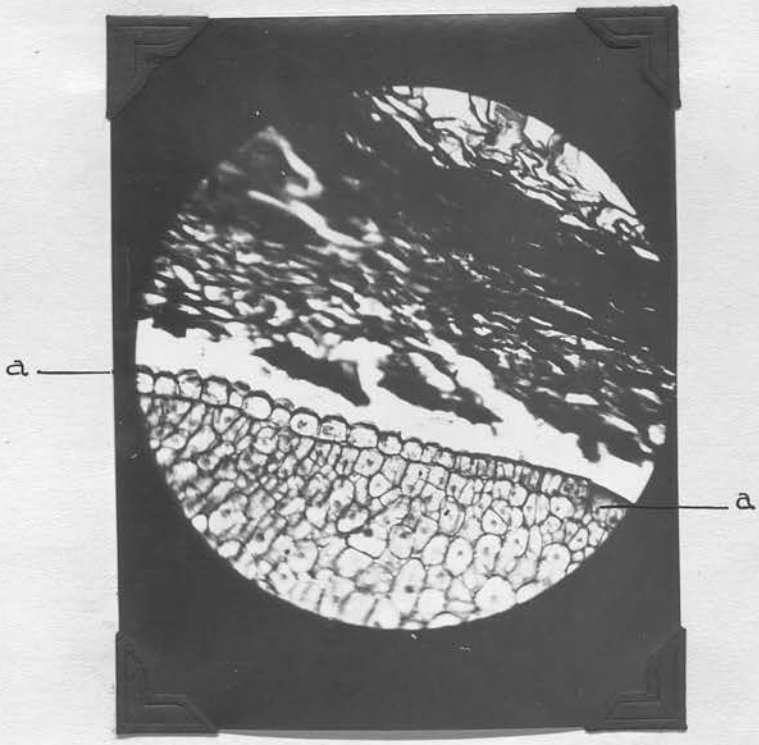
FIGURES 6 & 7.

Fig. 6. Transverse section of part of a bean seed. a. nutrient layer; b. crescent-shaped band; c. parts of cotyledon and hypocotyl. x 45.

Fig. 7. Transverse section of bean seed as in Fig. 6, more highly magnified, showing at a-a the papillate surface of the hypocotyl, and the smooth surface of the cotyledon. x 200.

FIGURES 6 & 7.



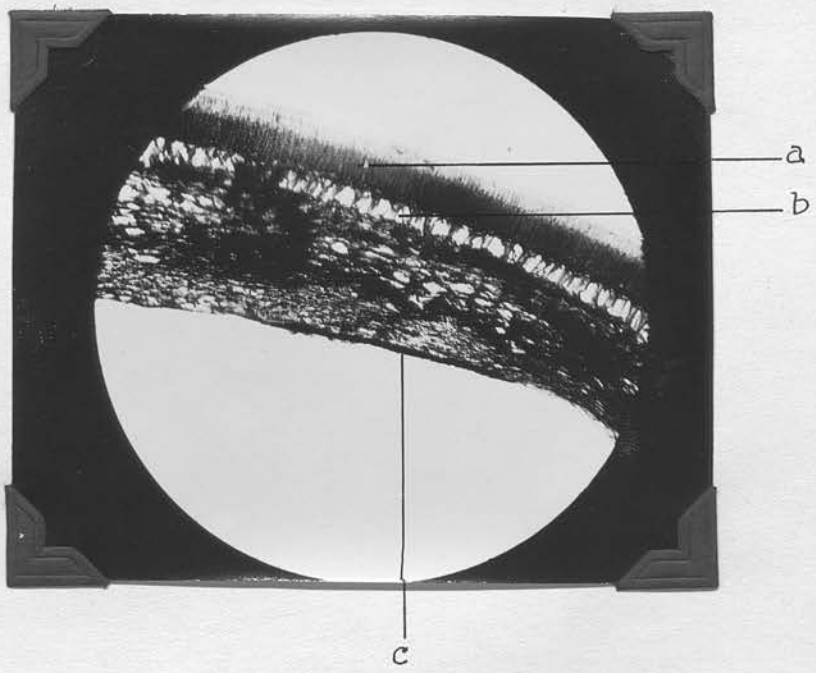


FIGURES 8 & 8a.

Fig. 8. Transverse section of bean testa through one of the crescent-shaped bands. a. palisade layer; b. hour-glass cells; c. crescent-shaped band. x 45.

Fig. 8a. Transverse section through a crescent-shaped band, showing the papillation. x 200.

FIGURES 8 & 8a.



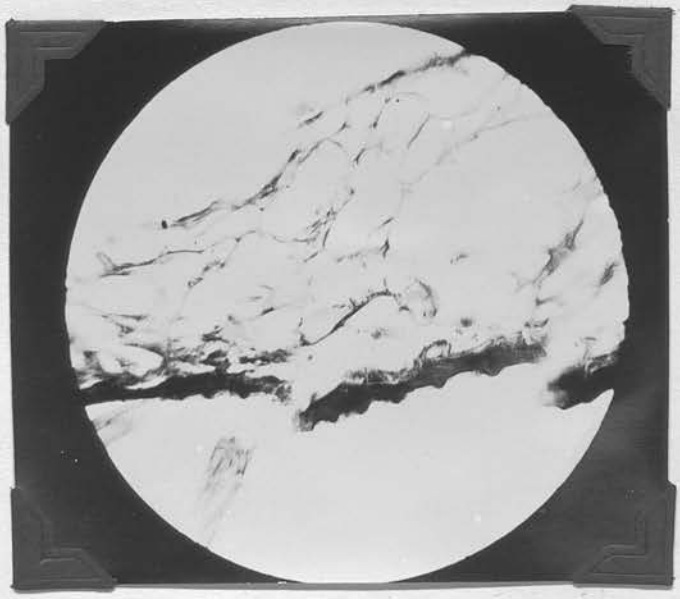


FIGURE 9.

Fig. 9. Transverse section through cotyledon and hypocotyl, showing orientation of the elongated cells extending from the "clamp connection".

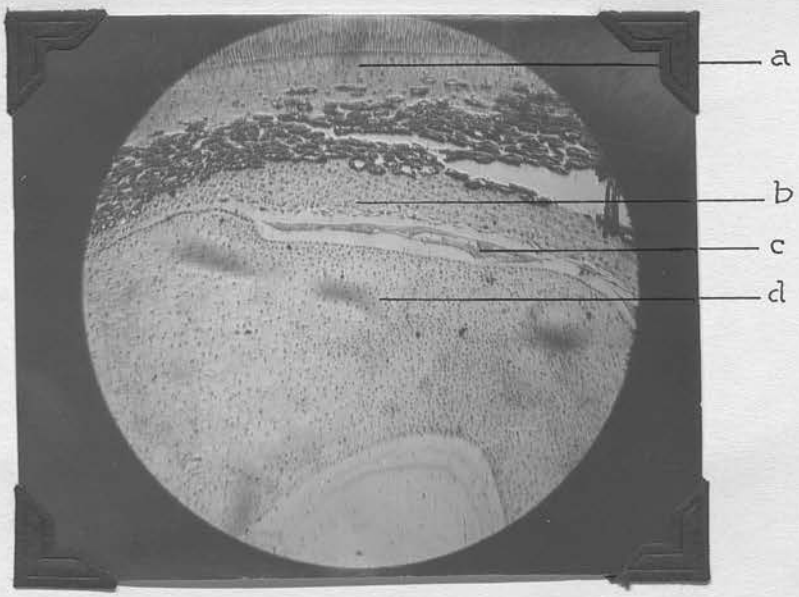
x 45.

FIGURE 9.

FIGURE IO.

Fig. 10. Transverse section through part of an immature bean seed in the region of a crescent-shaped band. a. palisade layer; b. nutrient layer; c. remains of endosperm; d. cotyledon and hypocotyl. x 200.

FIGURE 10.

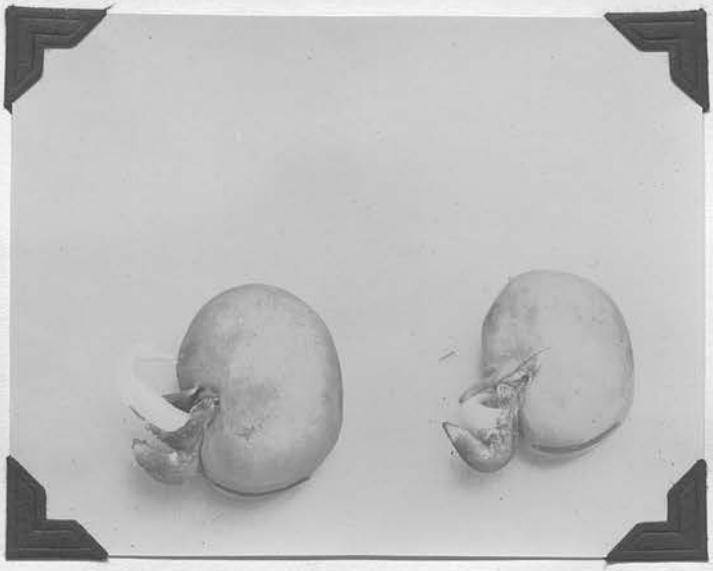


FIGURES 11 & 12.

Fig. II. Germinating beans showing the curvature of the radicle induced by the action of a saturated solution of Tolyl mercuri acetate.

Fig. 12. Longitudinal section of the radicle of a bean embryo, showing at a. the dead cells on the concave side. x 200.

FIGURES 11 & 12.



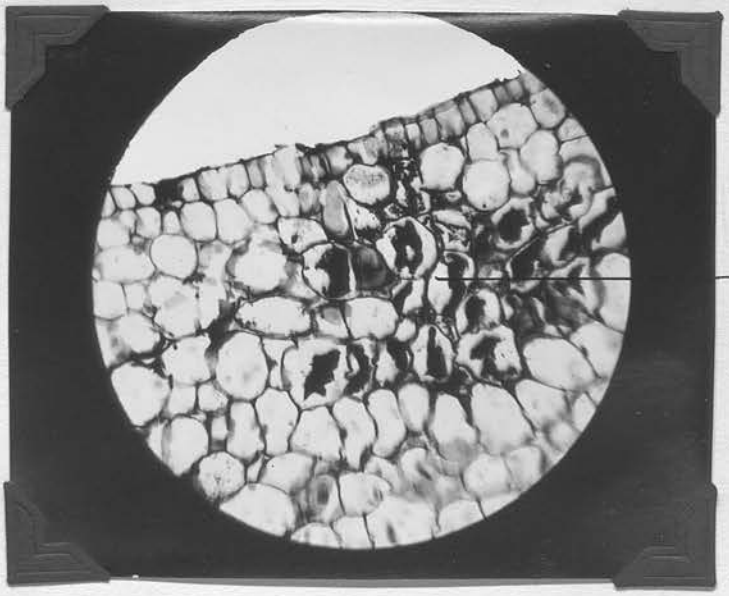


FIGURE 13.

Fig. 13. Germinating beans showing the effect, after eight hours, of a diluted solution of Tolyl mercuri acetate on the radicle.

FIGURE 13.

FIGURE 14.

Fig. 14. Germinating beans showing the effect of treatment with a saturated solution of Tolyl mercuri acetate. Bean Nos. 13, 7, 9, 5, reading from left to right.

FIGURE 14.

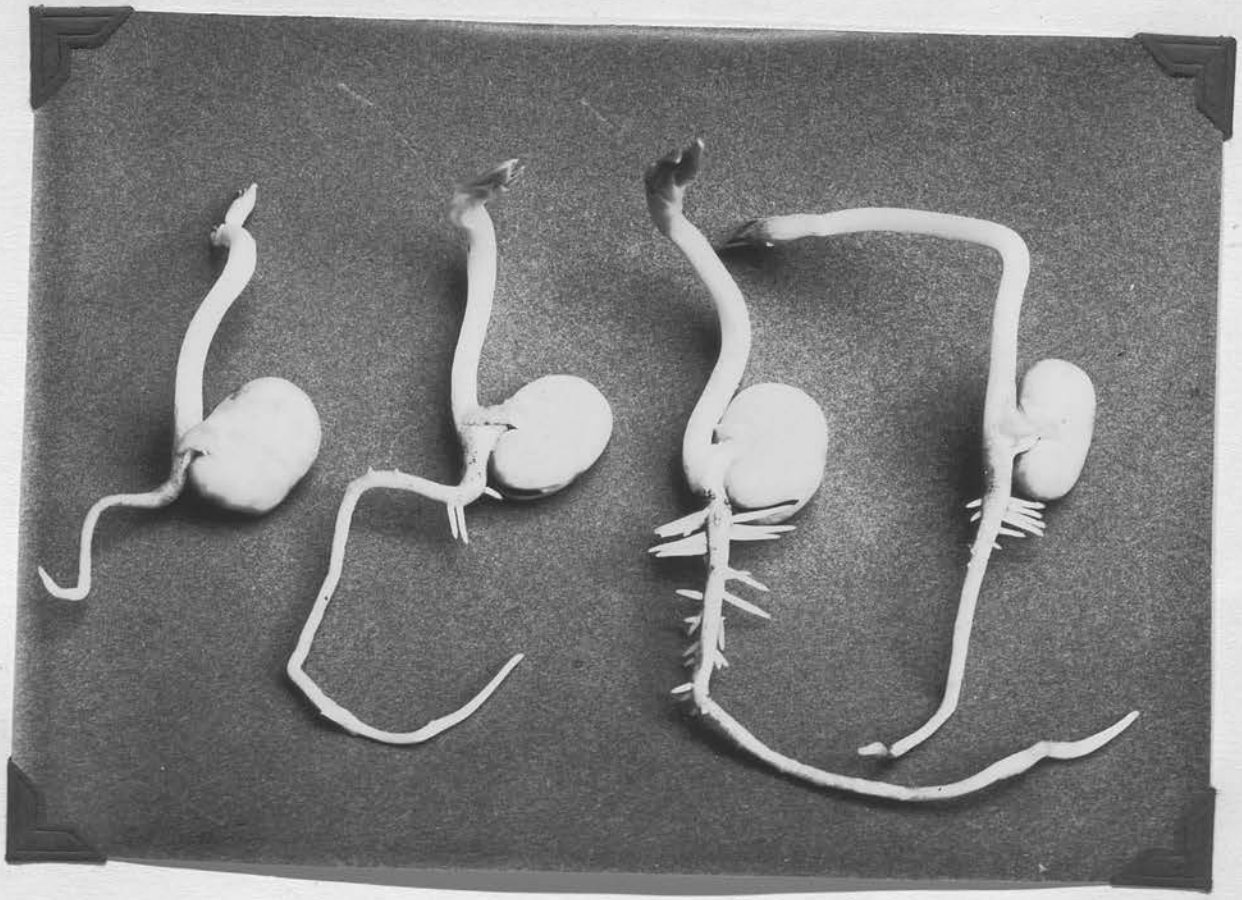


FIGURE 15.

Fig. 15. Bean seed with the micropyle, hilum and hilar bulges coated with paraffin wax.

FIGURE 15.

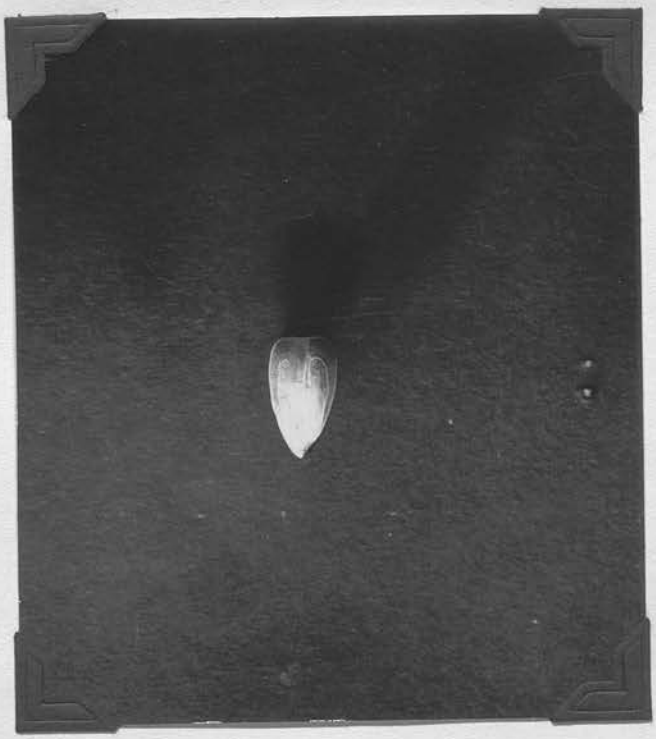


FIGURE 16.

Fig. 16. Germinating beans showing the effect of treatment with a saturated solution of Tolyl mercuri acetate (right), resulting in the production of a thin, whip-like root, compared with that of distilled water (left).

FIGURE 16.

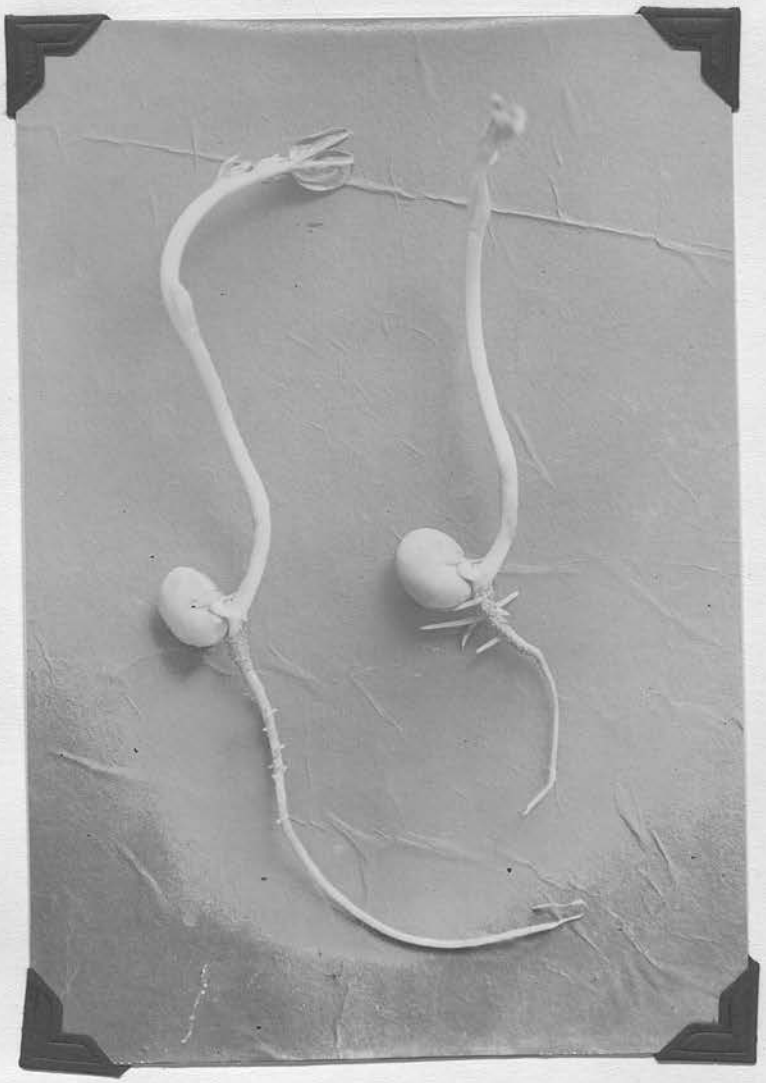


FIGURE 17.

Fig. 17. Germinating beans showing the effect of treatment with a saturated solution of Tolyl mercuri acetate (right), resulting in the production of a thin, whip-like root, compared with that of distilled water (left).

FIGURE 17.